2015

Examining Male Infertility; The Association Between Age, Environment, and Reproductive Success in Male Patients that have Participated in Assisted Reproductive Technology

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EXAMINING MALE INFERTILITY; THE ASSOCIATION BETWEEN AGE, ENVIRONMENT, AND REPRODUCTIVE SUCCESS IN MALE PATIENTS THAT HAVE PARTICIPAED IN ASSISTED REPRODUCTIVE TECHNOLOGY

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The School of Human Resource Education
and Workforce Development

by
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B.S., Louisiana State University, 2003
M.S., Louisiana State University, 2007
August 2015

The entirety of this work, its completion long anticipated is dedicated to my teacher and my friend, Robert A. Godke, PhD, Boyd Professor, Louisiana State University. More than a decade ago, I walked into his classroom armed with knowledge, and so I thought at the time, prepared for the reputation that preceded him. I could never have dreamt of the 'life' lessons that I would be carrying away with me all of these years later. I could not have and would not have wanted to complete this chapter of my journey without you. I will always have unparalleled pride in your life's work and will continuously be overwhelmed by your pride in mine.

"Even more important than ability to work, even more important than ability to fight at need, is it to remember that chief of blessings for any nations is that it shall leave its seed to inherit the land…. The greatest of all curses is the curse of sterility; and the severest of all condemnations should be visited upon willful sterility."

Theodore Roosevelt

ACKNOWLEDGMENTS

First and foremost, I would like to thank my Louisiana State University doctoral committee. Each member contributed instrumental attributes to my education, research, and to the completion of this dissertation for which I am grateful. I would like to sincerely thank Dr. Michael F. Burnett for graciously stepping in and providing direction to this portion of my academic career. I am extremely appreciative of his unparalleled guidance in the practices of the School of Human Resource Education and the procedures of social science research studies. Dr. Burnett has been nothing but encouraging, while allowing me the flexibility to pursue a combination of analytical and social science research. I am gratefully humbled that he had enough confidence in my ability to allow me the freedom to pursue this unique area of research. In doing so this enabled the resources available to define the spectrum of my dissertation work. I truly do not know where I would be in the pursuit of this degree at the present time, without the guidance of such an enthusiastic educator and dedicated Committee Co-Chairman. Dr. Burnett and his staff have shown great patience and continued guidance to ensure my success amid new protocols and procedures of an unfamiliar college of study. I would like to extend great thanks to Ann Harrington, Jody Bissett, and Linda Mumphrey for being nothing but welcoming and readily available since day one of my transfer to the college of human resources.

Heartfelt appreciation is extended to Dr. Kenneth R. Bondioli for serving on my doctoral committee as a representative member of my minor concentration in Reproductive Physiology. Dr. Bondioli has supported me in the pursuit of this

degree from the beginning. I am grateful to have had him as an irreplaceable member of both my master and doctoral committees. His expertise and guidance in the area of reproductive biotechnology have been and invaluable resource to me throughout my entire graduate education. Unarguably, Doc has the ability to always challenge me one step further in my thought process; helping to mold me into the student and professional that I am today. He has been unwavering in his availability to answer any and all of my inquires and I always looked forward to discussing unusual concepts with him.

Sincere thanks are also extended to Dr. T. Eugene Reagan for serving as the Dean Representative of my graduate committee. Under special circumstances, Dr. Reagan graciously gave of his time and expert guidance. I greatly appreciate his experience in the discipline of scientific research and scientific publication. It was a great advantage to have his assistance in the editing process of this document.

To Chancellor William B. Richardson, thank you seems like an inadequate expression to convey my gratitude for all of the time and guidance that you have provided me with other the past decade. I am grateful to have had Dr. Richardson not only as a mentor but as a friend. During this time, I have learned many great lessons from him on leadership and education, but the most important lesson that he has taught to me is balance. He has continually encouraged my educational and professional career, while also reminding me to share that enthusiasm in other important areas of my life. On rare occasions in our lives we come across individuals whose lessons are taught just as much by

watching their actions as hearing their words. Dr. Richardson is an example of the type of truly authentic teacher and human being that I continue to strive and mold my own personal character traits around.

I would also like to thank Dr. Richardson for serving as my doctoral committee Co-Chair. Additionally, I am grateful for being allowed the opportunity to pursue this degree while being employed with the LSU AgCenter. I would like to extend my thanks to Dr. Richardson's direct staff, Charlotte Smith and Simone Kramer for all of their assistance and concern.

In addition, I would like to recognize the Cooperative Extension staff in Pointe Coupee parish for truly being the best of the best. Mr. Miles Brashier, what we both thought was a pure coincidence of job placement turned into an irreplaceable experience. In coming home, I could not have picked a better environment to learn in or a more committed agent to learn lessons from that will last a lifetime. To Elaine Delatte and Mary Jarreau, thank you for pushing me, and for catching me, and for letting me vent. Your support and your friendship has been an unexpected blessing, which I have come to cherish. To all of my students in Pointe Coupee, thank you for showing me how much I love teaching and thrived at being a part of such a pivotal time in your lives.

A huge thank you is extended to my colleague and friend Carlos A. Guerrero, PhD, HCLD, ELD and Laboratory Director. Once again, I could not have completed this complex research without your assistance and unparalleled experience in the field of reproductive physiology. And I could not be more proud of your hard-earned success, while humbled by your continuous guidance. Thank

you, as well, to Jerald S. Goldstein, MD and Medical Director, for generously accommodating my research. I truly enjoyed the experience and learned a great deal from working in such an advanced clinical environment.

I would also like to thank all of my fellow graduate students for their camaraderie and encouragement, especially Ashley Wilson Mullens, Troy Autin, and the Repro Rangers. Just as importantly, I would like to express gratitude to my new family members. Bill and Jean Higgins, thank you for supporting me and encouraging me like one of your own. I would also like to extend heartfelt gratitude for providing me with a home away from home to complete my research. To the Jeters, Elliot, Ashley, Peyton and Ally and to Troy Higgins, along with your parents, thank you for your considerate interest and wonderful companionship at our family dinners during this lengthy journey.

To my enormous family, I would not be who I am today without each of you in my life. To our matriarch, Cora Lee Martin, I can never thank you enough for always being a constant in my life. You have been my rock and my biggest fan. Thank you for sharing so much of your life and your love with me. I have been truly inspired by your stories and your strength to succeed. In addition, I am grateful for your memories that connected me with my grandfather, Joseph Ivan Martin, and his time at LSU graduate school with the College of Engineering in the late 1930's.

I would also like to offer thanks to my aunt and uncle, Michel and Jim Beard, who have gone above and beyond to help me financially and spiritually throughout the years of my undergraduate and graduate education. I am truly

grateful for the open door policy to your house and to your hearts. Jim, I cannot thank you enough for steering me down this long and rewarding degree path. I will always be appreciative of your straightforward questions and, ironically, your advice to take risks. Ultimately, it was your no-nonsense question of; ‘why would you not,’ that gave me the grateful awareness of my opportunity and motivated me to pursue this PhD.

Annie Leonards Morrison, this work cannot be completed until I thank you. Thank you for always, always listening. Granted, most of the topics you may not have wanted to learn about but you did it anyway. I have so much gratitude for your friendship and your love. I will carry both with me for a lifetime. In a similar way, you will carry more knowledge on reproductive physiology than you ever cared to imagine.

Finally, I would like to thank my parents and my siblings for never once doubting my abilities. I express authentic gratitude to my father, Ted Glaser, for teaching me the principles of hard work and instilling in me the mindset to never quit. From him, my love of agriculture and the LSU AgCenter was born at a very young age. I will always be proud and grateful of my father’s work and personal introduction to this industry that has afforded me so many opportunities.

I offer sincere thanks to my dedicated brother, Jude Glaser, and to my loving sister, Kimberly Glaser. I would like to thank my bother for never doubting me, even to a fault, and for being my lifesaver on a few occasions throughout this arduous process. To my sister, I offer you the sweetest thanks for constantly checking on me and expressing your pride in me. You continue to show true

strength and grace in the face of challenges and you remain my inspiration to succeed for us both.

While my dad taught me irreplaceable tools to success, my mother showed me how to practice them. I offer endless thanks to Jackie ‘Jacq’ Martin. Without her presence this accomplishment in my life would have never been possible. She was, and always will continue to be my first supporter, my first friend, and my first teacher. My mother instilled in me a love affair with reading and a lifelong thirst for knowledge. More importantly she raised me to believe that anything was possible; my world was truly limitless. Without being introduced to such an influential mind set I would never have dreamt of pursuing such a lofty goal. I will never be able to thank her enough for the foundation that she provided me with and for her financial and loving support; especially when she did without to help me move forward. I owe her my education, my life; everything.

Lastly, I would like to thank the newly created Glaser Higgins household. Excluding me, that only leaves two other members of our little family, my husband, Jason Albert Higgins and our thirteen year old weimaraner-boxer mix, Ellie. I cannot thank the Lord enough for putting me in the exact right place, surrounded by so much love, to finally complete this longtime goal. Ellie has been my constant writing companion through it all. Some may think I have gotten a little carried away, but the truth is that I could not have made it through this process without her calming and consistent presence in those long hours. She literally stayed by my side, sitting at my chair or under my desk as I wrote this

entire dissertation. I will have a lifetime of special gratitude for her loyalty and for her unconditional love.

I am certain very few people will ever truly understand the sacrifices that were made for the publication of the following pages. However, I intend to continuously thank my incredible husband for being the first one on that list. Jason Higgins, I will be forever grateful for your understanding. The hours of commitment that it took, to not only complete this degree, but to complete it the right way. Thank you for believing in me when I was too exhausted to believe in myself. Thank you for your attempted understanding of my research, at least most of the time, and for believing that I am too smart for my own good. Thank you for your patience in me. I have slowly come to realize that no matter how independent I think I am, my sacrifices are now your sacrifices and my endeavors are your endeavors. Above all, I want to thank you for your happiness in my victories and the incomparable joy that you bring to those shared moments. I am already wealthy beyond my imagination because I have been blessed with gifts that money cannot buy. I love you.

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ABBREVIATIONS USED IN THIS DISSERTATION

AI – artificial insemination
ART – assisted reproductive technology
βhCG – Beta Human Chorionic Gonadotropin
BMI – body mass index
CDC – Centers for Disease Control and Prevention
ºC – degrees Celsius
DNA – deoxyribonucleic acid
E_2 – Estrogen
ET – embryo transfer
FSH – Follicle-Stimulating Hormone
GnRH – Gonadotropin-Releasing Hormone
HED – high-energy diet
hCG – Human Chorionic Gonadotropin
ICSI – intracytoplasmic sperm injection
ILCP – infertility length with current partner
IUI – intrauterine insemination
IVF – in vitro fertilization
kg/m^2 – kilogram per meter squared
µm – micrometers
mg – milligram
ml – milliliter
OBGYN – Obstetrician Gynecologist
PHSG – Pregnant Human Serum Gonadotropin
LH – Luteinizing Hormone
ROS – reactive oxygen species
SAM – senescence accelerated mouse
SART – Society for Assisted Reproductive Technologies
Sp-AR – spontaneous acrosome reaction
SSCs – spermatogonial stem cells

ABSTRACT

As the number and age of human couples turning to assisted reproductive technology (ART) continues to increase, it is essential for clinicians to understand infertility threats related to both female and male patients. The objective of this study was to evaluate the association between age, environment, and reproductive success in male patients having participated in assisted reproductive technology. In corresponding experiments, male infertility variables such as; age, lifestyle exposures, body mass index (BMI), and infertility length with current partner (ILCP) were investigated. A retrospective collection of clinical male patient data from 2011 to 2014 was evaluated. Thirty-five variables were collected from an original sample of 132 patients and correlated for relationships related to male fertility. A negative relationship was observed between pregnancy and male age, IVF pregnancy and male age, male age and semen volume, and male age and semen progressive motility. A negative correlation was also revealed among alcohol usage and semen volume and alcohol usage and total motile sperm/specimen. Additionally, a positive correlation was observed between ILCP and percent normal semen.

The goal of the following study, the clinician survey, was to evaluate and compare differences in opinions. Questions pertained to male infertility factors and fertility clinic practices. Clinicians responded with the following opinion rates; 67.9% felt semen analysis was an effective predictor, 32.7% reported no idea if DNA fragmentation was a predictor, 58.5% were in agreement that male age had somewhat significance, 80.1% responded that genetics and/or epigenetics

displayed somewhat or significant influence (41.5% and 39.6%), 58.5% believed male exposure/environmental factors displayed significance, 53.9% felt access to more male information would enable better care. The most commonly seen descriptive variable clinicians reported was ILCP (70.8%), the most important semen characteristic was sperm count (84.6%), the most commonly seen urological variable was vasectomy (77.8%), smoking was the most commonly seen environmental exposure (74.5%), and medication use was the most commonly seen medical variable (84.8%). Clinicians described that 39.1% of patient charts were <25.0% completed and 63.0% of clinicians acknowledged that the industry was not providing adequate male reproductive information to infertility patients.

CHAPTER I: INTRODUCTION

Rationale

As the 20th century transformed into the 21st, a cultural shift in society identified a need and an occasion for increased research on the effect of reproductive threats associated with advanced paternal age and paternal environmental exposure factors. Both of which may contribute negatively to male reproductive success. Despite a number of maternal studies in the second half of the 20th century, research investigating the role of paternal age in adverse birth outcome is limited. Consideration and identification of specific paternal factors will aid in increased semen quality, increased fertility rates, increased pregnancy rates, and decreased number of still births and fetal abnormalities.

The past few decades have revealed an increase in the amount of human couples turning to assisted reproductive technology (ART) procedures. Research has shown that this increase is due in large part to the newly established trend of postponing childbirth. Couples in the United State have progressively delayed starting families because of societal changes, cultural expectations, career aspirations, and financial situations.

This rise in ART patients can largely be attributed to the increased number of infertility treatments in older patients. The use of infertility treatments has risen dramatically in the past 20 years; between 1996 and 2003, the number of human ART cycles performed in the United States nearly doubled from 64,681 to 122,872 (Institute of Medicine (US) Committee on Understanding Premature Birth and Assuring Healthy Outcomes, 2007). In 2013, Chandra and colleagues

clarified that, although treatment use has been raised, infertility rates have actually decreased from 8.5 to 6.0% of married women between 1982 and 2010. The authors explained that because couples are waiting longer to start families, fertility issues or delays may be involved with the increase in infertility treatments but should not be associated with a rise in infertility rate.

Regardless of the cause of this influx in infertility treatments, the association of increased maternal age and the risk of higher reproductive failure has been well established. Conversely, the link between paternal age and birth outcome has received far less attention. There are several factors at fault for the hindrance of studying paternal age.

A large amount of attention has traditionally been focused on maternal influences on fetal growth. In 2008, Chen and colleagues pointed out that, to date, maternal influence has universally been considered of more importance than paternal influence. Additionally, research has demonstrated that the biological father is unknown in some cases, further hindering the investigation of paternal effects. In the 2006, paternal age was missing from the U.S. vital statistic records for 39.0% of unmarried women, but only 0.4% of married women (Basso and Wilcox, 2006). Furthermore, from an epidemiological standpoint, it is more convenient to study the effects of maternal factors on birth outcomes. Pregnant women generally make frequent prenatal care visits to their physician or hospital, thereby facilitating the collection of information on maternal characteristics that may affect birth outcomes (Chen et al., 2008).

Recent research has revealed that as males age and are exposed to detrimental factors semen quality can decrease. Although it is possible for men to father children into old age, the genetic quality of sperm, as well as its volume and motility, typically decrease with age and negative environmental factors. Therefore, it is important to note that currently semen quality is the primary measurement of the ability of sperm to accomplish fertilization. It is the sperm cells in the semen that are of importance, and therefore semen quality involves both sperm quantity and quality. Decreased semen quality is a major factor of male infertility.

Notably, fifty percent of the embryonic genome is derived from paternal deoxyribonucleic acids (DNA) in the sperm cell. In contrast to paternal DNA contribution to successful fertilization, increased sperm DNA damage can adversely affect embryo quality. These detrimental effects can be observed starting at day-2 of early embryonic development and can continue to be detected following embryo transfer; resulting in reduced implantation and pregnancy rates (Simon et al., 2014).

Statement of the Problem

Regardless of age, human sperm samples are very heterogeneous and include a low amount of truly functional gametes (Sousa et al., 2011). Although all sperm may look the same to a casual observer, human ejaculates are varied, and subpopulations of sperm with distinct biochemical and physiological characteristics can be identified in every sample (Sousa et al., 2011). It is actually believed that only a very small percentage of sperm is able to achieve

fertilization (Holt and Van Look, 2004; Holt, 2005). This produces a challenge for the accumulation of substantial and consistent data on human male sperm parameters. Exposing the heterogeneous nature of human sperm as one of the major challenges researchers are facing in the industry today when trying to better characterize and isolate a particular useable subpopulation.

Although researchers, such as Sousa et al. (2011), have demonstrated advanced fractionation techniques to obtain subpopulations with improvements in certain sperm parameters, a subpopulation including only fertile sperm has never been isolated. This is mainly due to the fact that we are still not able to completely describe what makes a competent spermatozoon (Sousa et al., 2011).

Research has shown that paternal semen influences on reproduction are quite important. It is believed that approximately half of the couples that turn to assisted reproductive techniques do so because of male infertility factors. Therefore, due to the increasing proportion of couples participating in ART procedures, predicting outcomes is of ever increasing importance. Although researchers have established a number of unfavorable factors from maternal influence, male factor infertility is still relatively understudied. Scientists have increasingly acknowledged that male factors provide a significant amount to the successful treatment of an infertility couple. Since pregnancy rates following in vitro fertilization (IVF) are still quite low, prognostic information for both the male and female is very helpful in making clinical decisions (Brincat et al., 2014).

In 1993, Giwercman et al. reported data that clearly indicated semen quality had markedly decreased during the period 1938-1990, and concurrently the incidence of some genitourinary abnormalities including hypospadias, maldescent, and cancer had increased. Researchers explained that such a significant increase in the occurrence of gonadal abnormalities over a relatively short period of time was more likely to be due to environmental factors rather than genetic factors.

Generally, it has been believed that pollution, smoking, alcohol, and sexually transmitted diseases play a role in male infertility. In addition, researchers have proposed that increased male age, body mass index, previous illnesses, medication, steroidal and hormonal usage, and trauma to the testicles have also contributed to the decrease in quality of a man's sperm. As more information is being established on how the effect of male age and the environment have contributed to sperm quality, many patients want to know how to 'fix' this problem.

While it is still assumed that the medical risks may be smaller for older fathers, the increase in couples becoming parents later in life emphasizes the issue that male age and exposure should be taken seriously. According to a 2014 Centers for Disease Control and Prevention report, approximately six million couples in the United States are infertile. Research has shown that in about one third to one half of these couples, a male sperm factor is partially or completely responsible.

In 2004, it was reported that 24 in every 1,000 men aged 40 to 44 fathered a child (Centers for Disease Control and Prevention, 2014). That number was up almost 18.0% from the decade before. Meanwhile, only three out of every 1,000 men aged 55 and older had fathered live births. As men are attempting fatherhood later in life, 1.2 million men seek help for infertility, and those are just the cases that are reported. Fifteen percent of these men are accurately diagnosed with male factor infertility using a semen analysis (Guzick et al., 2001).

Chandra and colleagues (2013) reported that among men from 2006 to 2010, some form of infertility was reported by 9.4% of men aged 15 to 44 and by 12.0% of men aged 25 to 45. These statistics demonstrate that as couples are waiting longer to conceive advanced age should be a concern for both parents. These findings demonstrate the importance of knowing the mutual contributions of both the male and female patient. However, that information is not yet widely recognized outside of the human infertility industry. To date, statistics for ‘infertility’, listed online by the CDC have overwhelmingly contained female fertility problems and only minimally addressed male factors.

The last century endured witness to science curriculums educating millions of students that there is not an identified specific male age associated with the senescence of reproduction. In recent years, researchers have found some success in demonstrating a gradual decline in male fertility as age increased. Different from the acute onset of menopause in females, male

infertility research demonstrated a gradual change in the reproductive system associated with advanced age.

Exact biological mechanisms of reproductive alterations in older males have been difficult for researchers to identify and vary among individuals. Consequently, there is little being done to replace the previous ideas of limited male reproductive influence with the education of this advanced theory. In fact, extreme cases of advanced male reproductive success are actually being more highly publicized; not just from media outlets but from boasting physicians as well. As a result, the small amount of data on the detrimental effects of advanced male reproductive age, have been unsuccessful in protecting outrages cases from continually being pursued by some male patients and some physicians.

In addition to male age, infertility patients should receive just as much information on the further risks of male factors as they receive on female factors. Many research studies show that this is not the case. Two obvious explanations for this problem are the lack of male patient data reported to be properly addressed and the lack of patient knowledge and/or minimization of the male role on human reproductive success.

When most people hear the term preconception health, they only think of the female. However, preconception health is important for the male, as well. There are things men can do to improve their own health, as well as the health benefits of their female partner and future children. Ethically, it is in the best interest of the industry and the patients to give as much information as possible

on male infertility factors. Additionally, patients need to be educated on the benefits of participating in male preconception health.

Just as overall health, lifestyle, and age can affect female reproductive success, the same factors have been shown to affect male semen quality and reproductive success. Male gametes are extremely delicate and susceptible to factors that affect normal semen production. By reducing the health and/or number of sperm male reproduction can be affected. Also reported to impact sperm production is; heavy alcohol use, drug use, advanced age, and environmental toxins. Health problems such as mumps, serious conditions like kidney disease or hormone problems, medications, and radiation treatment and/or chemotherapy for cancer (Office on Women's Health, 2012) can initiate abnormal sperm production. In addition, obesity among men has been associated directly with increasing male infertility (Sallmén et al., 2006; Frey et al., 2008).

Adding to the problem, a 2014 Center for Disease Control and Prevention consensus illustrated that the percentage of male data reported to analyze was much less than the amount of female data available. The group suggested that the lower number of available data was a reflection of the difference in reporting from the male perspective. Clinicians are currently observing the same problem in their private practices. Lack of completed male data reports can hinder treatment practices.

Regardless of the lower percentages of infertility service usage actually reported among men, similar infertility associations have been identified as those

seen in women, such as; advanced male age, marital status, and other demographic characteristics (Centers for Disease Control and Prevention, 2014). This further demonstrates the need to convey male infertility factors to ART couples and encourage proper male patient record documentation.

For both male and female patients, advanced age is a reality when having children later in life. As with most things in life, there are pros and cons of being older parents. However, many professionals in the industry believe that a double standard has come into play for male versus female patients. History shows that on average females live longer and that the burden of most treatment and follow up care is carried by the female patient.

When examining the ethics surrounding the gain in new data connected to male infertility, other issues have begun to arise. Many infertility clinics implement female cutoff ages. However, this same standard does not apply for a comparable percentage of male patients. Even as knowledge in this area is increased, the medical and ethical concerns should remain the same. The overall welfare of the offspring and the proper treatment of both the male and female patient are of top concern.

The results of the online clinician survey, created by the researcher, further demonstrated the various discrepancies in human fertility treatment. Since there is little regulation beyond quality control in fertility laboratory settings, there is currently a considerable amount of treatment variations. Although much may be miniscule, when it comes to determining the best application practices any differences can create controversial results and recommendations.

Significance of the Study

The researcher, in conjunction with an outside private facility, has the technology and resources to establish specific factors which will then become indicators for further research. This study will create new, available data to be used by the human fertility industry.

Over the past decades, the majority of infertility research data obtained has mainly been focused on female factors. As previously mentioned and to be discussed in further depth in Chapter II, couples are waiting longer to start their families. This trend has created an increased need to focus additional research on male infertility factors. As we gain more knowledge on the significance of male factors it is necessary to present these findings and to further the research in these areas to provide better treatment for infertility couples.

Data obtained from the completion of this study is intended to provide useful information to physicians, physiologists, clinical staff, administrators, policy makers, health care providers, sperm donors, and infertility couples. The findings can be beneficial to researchers and human infertility professionals, as well. The data can serve as a resource for both the clinical and investigative systems as they adapt their practices to meet the personal needs of individual infertility couples. Finally, couples facing infertility can gain meaningful knowledge on lifestyle factors and lifestyle changes that can improve their fertility success rates and enhance their infertility treatment.

CHAPTER II: REVIEW OF LITERATURE

Reproductive Physiology

For the human species to continue surviving, it is necessary to produce fertile offspring. This is necessary to continue the existence of the species and to pass on genetic information from generation to generation. The process is accomplished through normal reproduction. Organisms generate new individuals of the same kind through a sexual or asexual process. Human reproduction is any form of sexual reproduction resulting in the conception of a child.

In Homo sapiens, the natural capability to produce offspring is characterized as fertility. Under both genetic and environmental control, fertility is influenced by male and female gamete production, fertilization, and gestational term. The natural capability of a couple to produce live offspring is considered successful reproduction; therefore a lack of success is considered infertility.

Research has demonstrated a number of biological and environmental factors that can possibly lead to the infertility of females, males, or both members of the couple. In humans and other similar mammalian species, to become pregnant is a complex processes that requires many balancing parts. Any step that is disrupted throughout the process may lead to an unsuccessful reproductive experience.

Assisted Reproductive Technology, known as ART, are techniques used to aid in achieving reproductive success. Artificial methods to obtain human pregnancy involve the treatment of human oocytes, sperm, and/or embryos. However, the availability of this assisted therapy has not always existed and is

considered a young field in the discipline of science. There is much research left to be done and an endless amount of additional knowledge to be gained.

History of Reproductive Physiology

The birth of modern reproductive and developmental biology took place as early as the 17th century. Spermatozoa were first reportedly discovered, by Anton Leeuwenhoek (1632-1723), using a homemade lens magnified 300 fold. In 1780 scientist Lazzaro Spallazani performed the first recorded successful artificial insemination (AI) by developing a technique to artificially inseminate a dog.

By the 19th century, significant progress in the scientific knowledge of mammalian reproduction and development was being reported. Important contributions to this progress were the discovery of the ovum by Karl von Baer (1792-1876). His observations of the stages of embryogenesis, led to the remarkable descriptions made by Edouard Van Benden (1845-1910) of oocyte development in rabbits and bats (see Alexandre, 2001) half of a century later. Albert Brachet furthered these advancements by his report of keeping a rabbit blastocyst alive and developing in blood plasma for 48 hours outside of the mother's body (1912, 1913).

The second half of the 20th century saw an advancement in mammalian embryology when a handful of scientists such as; Biggers, McLaren, and Whitten reported the development of murine oocytes in a chemically defined culture medium (McLaren and Biggers, 1958; Whitten, 1957). The successful production of murine offspring, after the transfer of in vitro cultured embryos was reported by

McLaren and Biggers (1958). This development led to first the successful in vitro offspring of several species, including humans.

Mid-decade, Austin (1951) and Chang and Pincus (1951) reported a major technological barrier to in vitro fertilization (IVF); the process of sperm capacitation. Sperm capacitation normally occurs in the female reproductive tract and renders sperm cells capable of fertilizing ova. However, in 1954, Thibault and colleagues successfully accomplished IVF by using sperm cells recovered from the uterine milieu of mated does.

In 1959, Chang reported the birth of the first live mammalian, a rabbit, following in vitro fertilization, thus opening the way to assisted procreation. Finally, by 1975, it became evident that ejaculated rabbit spermatozoa could in fact be in vitro capacitated, enabling in vitro fertilization, and the development of resulting embryos into live offspring (Bracket and Oliphant, 1975).

In 1978, biologist Robert G. Edwards and gynecologist Patrick Steptoe produced the first human baby by in vitro fertilization (Steptoe and Edwards, 1978). In February of 1979, the researchers presented their results to the Royal College of Obstetricians and Gynecologists in London. With their study finally published in 1980, Edwards and colleagues described that the intentions of their research were to recover pre-ovulatory oocytes by laparoscopy, fertilize them in vitro using spermatozoa from the husband, grow the embryos in culture three or four cleavage divisions, and then place them in the mother's uterus (Edwards et al., 1980). This paper was the first of its kind in a series of papers presenting the researchers' observations, methods of treatment, and results. Many of the

applied human embryo culture protocols and resulting implantation rates have remained relatively the same as those described by Edwards and collaborators in 1980.

Human Reproduction

In humans, the processes of ovulation and fertilization must occur within a specific time frame inside of the female reproductive tract to achieve conception. The female ovaries cyclically develop and release a mature, competent oocyte through ovulation. A complicated process that involves purposeful destruction of follicular tissue, ovulation is initiated by a hormonal surge to expel an unfertilized oocyte (Senger, 1999). In 1981, Wright and Bondioli described the series of fertilization events in specific order; (1) contact with and penetration of the cellular investments of an ovulated oocyte by a spermatozoon; (2) penetration of the oocyte's zona pellucida; (3) fusion of the spermatozoon and oocyte external membranes; pronuclei fusion (syngamy); and (4) alignment of their respective chromosomes on the first cleavage spindle. Ultrastructure studies have documented each of these physiological events (Austin, 1968; Bedford, 1970; Zamboni, 1971; Gould, 1975; Gwatkin, 1977).

The primary structures of in the human female reproductive tract include the ovaries, oviducts, uterus, cervix, vagina, and external genitalia, all of which play a vital role in maintaining and sustaining gestation. These structures are described in great detail in P. L. Senger's 1999 textbook, Pathways to Pregnancy and Parturition. In the same publication, Senger (1999) outlined the primary

components of the male reproduction system as well. These male reproductive structures will be described in detail in the next section of this review of literature.

In humans, the moment of conception begins at fertilization, with the fusion of viable male and female gametes to produce a new organism. Human fertility is dependent on a number of factors; age, nutrition, sexual behavior, culture, instinct, endocrinology, timing, economics, way of life, and emotions, to achieve each successful conception.

Male Reproduction

Concern has increased on the impact of the environment on public health, including reproductive ability (Carlsen et al., 1992). Arising controversy from separate reviews have claimed that the quality of human semen has declined (Nelson and Bunge, 1974; James, 1980; Leto and Frensilli, 1981; Bostofte et al., 1983; Osser et al., 1984; Menkveld et al., 1986; Murature et al., 1987; Bendvold, 1989; Li et al., 1991; Swan et al., 1997; Swan et al., 2000). However, only little attention has been invested in these warnings, possibly because the suggestions were based on data from selected groups of men recruited from infertility clinics (Bostofte et al., 1983; Osser et al., 1984; Menkveld et al., 1986; Bendvold, 1989), from among semen donors (Leto and Frensilli, 1981), or from candidates for vasectomy (Nelson and Bunge, 1974).

However, this specific selection of male samples is intentional because of the lack of availability on male infertility information in other areas. The limited sample groups mentioned in the previous studies are actually the most assessable groups from which to gain research on male infertility, even though

that information is still incomplete as compared to available research on female infertility. It is worthy to note, however, that in 1987 the World Health Organization reported that the lower reference value for a 'normal' sperm count has changed from $60x10^6$/ml in the 1940's (Hammen, 1944; MacLeod and Heim, 1945) to the percent value of $20x10^6$/ml (World Health Organization, 1987).

In 1992, Carlsen and collaborators concluded that data on semen quality collected systematically from reports published worldwide indicated clearly that sperm density has declined significantly during 1938-1990, although they could not conclude whether or not the decline is continuing. Simultaneously, the group pointed out that the incidence of some genitourinary abnormalities; including testicular cancer and possibly maldescent and hypospadias have increased. The researchers (Carlsen et al., 1992) inferred that such remarkable changes in semen quality and the occurrence of genitourinary abnormalities over a relatively short period were probably due to environmental rather than genetic factors. Furthermore, the researchers proposed that some common paternal influences are assumed to be responsible both for the decline in sperm density and for the increase in cancer of the testis, hypospadias, and cryptorchidism (Carlsen et al., 1992).

The male reproductive system is regulated by interplay between the nervous system, the endocrine system and the reproductive gonads. The hypothalamus is the neural control center for reproductive hormones and the endocrine system relies on these hormones to cause responses in target tissues. An important regulator of the spermatogenic process, involving the interplay

between the hypothalamus, pituitary and testicles, is the hypothalamus pituitary-axis (HTP). The presence of specialized neurons in the hypothalamus release the gonadotropin-releasing hormone (GnRH) in a pulsatile manner, stimulating the production of the two pituitary hormones, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which are the functional link between the brain and the testes. FSH targets the Sertoli cells which play a major role on sperm germ cell development and LH acts on the Leydig cells stimulating testosterone production.

To understand the importance of semen quality, it is essential to understand that the male reproductive system is made up of a number of components that must all be activated at the appropriate time. In addition, at any step along the spermatogenesis process, harmful factors can hinder normal sperm production. The key components of the male reproductive system described by Senger (1999) are as follows:

Spermatic Cord

The function of the spermatic cord is to provide vascular, lymphatic and neural connection to the body, to provide the countercurrent heat exchanger and to house the cremaster muscle (Senger, 1999). All of these components are essential in the production of viable spermatozoa by preventing disruptions from affecting the function of the testes.

The spermatic cord extends from the body cavity into the scrotum and attaches to the dorsal pole of the testes, suspending the testes in the scrotum. Abnormal sperm production or sperm function is correlated with the anatomical

function of the spermatic cord and can account for a majority of male infertility problems. Undescended testicles, genetic defects, health problems including diabetes, prior infections such as mumps, trauma or prior surgeries on the testicles or groin inguinal region can all affect sperm production (Mayo Clinic, 2014).

The design of the spermatic cord is to create an environment that lowers the temperature of the venous blood traveling through the testicular veins, and subsequently the testicular arteries. As previously mentioned, the spermatic cord suspends the testis in the scrotum, allowing for venous blood to be cooled by direct heat loss through the skin of the scrotum. Within the spermatic cord a network of veins are tightly intermingled with a highly coiled spermatic artery. Through countercurrent heat exchange, the warmer arterial blood temperature is cooled by the lower venous blood temperature, maintaining testicular temperature at about 4 to 6ºC cooler than the rest of the male body (Senger, 1999).

Maintenance of low testicular temperature is imperative for spermatogenesis to occur. Any disruption or modification of this cooling system will severely compromise; if not completely suppress sperm production. A number of male infertility problems can originate from increased testicular temperatures, whether it is from the male's occupation, a sedentary lifestyle or a high fever, increased testicular temperature can damage sperm cells.

In his textbook, Senger (1999) explained that researchers reported exposure of the scrotum to hot temperatures for periods of 16 hours a day did not

influence the number of spermatozoa. However, a reduction in motility and percentage of live spermatozoa occurred when the testes were heated for only eight hours per day.

In addition, frequent exposure to heat, such as in saunas or hot tubs, has been shown to elevate testicular temperature and impair sperm production (Shefi et al., 2007). The groups' findings also demonstrated that after heat exposure semen quality varied biologically among individuals and could actually be reversed in some infertile men (Shefi et al., 2007). Unfortunately, the results of common male exposure studies, such as this one, remain unknown to the majority of the population. This critically support the fact that it is important for male partners to understand the effects of heat exposure as it relates to successful pregnancy rates.

Testes

The human male gonads have two important functions: (1) they produce the hormone testosterone, which produces the deep male voice, beard, and sex drive; and (2) they produce sperm (National Institute for Occupational Safety and Health, 1996). Serving as the manufacturing and assembly site for the process of spermatogenesis, it is in the testes where male gamete production takes place. The process of spermatogenesis summarizes all events that transform basic spermatogonia into highly specialized mature spermatozoa within the male gonads (Wistuba et al., 2007). Before a gamete can leave the testis, it passes through several stages of maturation. The process includes mitotic multiplication and propagation of the spermatogonial stem cells (SSCs), meiotic recombination

of genetic material and testicular maturation of spermatozoa (Ehmcke et al., 2006).

Mammalian testes interplay between three systems within the male body, the reproductive system, the nervous system and the endocrine system. Considered the primary reproductive organ in the male, producing both spermatozoa and the androgen testosterone, the testes consist of two major compartments, the seminiferous tubules and the interstitium.

The seminiferous tubules are the place of spermatogenesis and are part of the tubular compartment of the parenchyma, a cellular mass of connective tissue, of the testicles. The seminiferous tubules also produce a fluid, which serving as a vehicle in which spermatozoa are suspended and facilitating in their removal from the testes (Senger, 1999). The interstitium is responsible for blood supply, immunological responses and contains Leydig cells that mediate endocrine signals of the pituitary to the testis and back to other body functions (Wistuba et al., 2007).

In the fully developed mammalian testis, the majority of undifferentiated cells of the germ line are type A spermatogonia (Wistuba et al., 2007). This population of cells also includes the SSCs. Wistuba et al. (2007) described these as the most important cells for spermatogenesis because their task is to provide both self-renewal of the SSCs and type B spermatogonia . Type B spermatogonia differentiate and develop into primary spermatocytes. The primary oocytes undergo meiosis and secondary spermatocytes are produced

with reduced genomic content. Another genomic reducing step next leads to the development of haploid spermatids (Wistuba et al., 2007).

In addition to the structural mechanisms of the testes, which produce a protein called testes determining factor, the convoluted seminiferous tubules are responsible for the production of Sertoli cells. The basic function of the Sertoli cells is to nourish the developing sperm through the various stages of spermatogenesis. Activated by FSH, Sertoli cells are specifically located in the only place in the testes where spermatozoa are produced. Anchored to the basal compartment of the seminiferous epithelium, Sertoli cells surround the developing population of germ cells. Here a blood-testis barrier is formed from the peritubular cells surrounding the seminiferous tubule and the Sertoli cell junctional complexes to prevent immunologic destruction of developing germ cells.

Considering the primary role that the testes play in male reproduction, there are a number of associated malfunctions that can contribute to infertility. Enlarged veins in the testes can increase blood flow and heat, affecting the number and shape of sperm. National Institute of Occupational Safety and Health (2006) described how reproductive hazards can actually reduce the number of sperm produced and/or cause damage to sperm morphology and motility. Just this year, Lotti and colleagues (2015) published a study in which infertile males smokers showed lower ejaculate and ultrasound-derived seminal vesicles volume in the testes, despite higher testosterone levels, when compared with non-smokers. Similarly to women, damage related to cancer and its

treatments, including radiation or chemotherapy can be detrimental to male fertility. In a bi-gender evaluation, Müller (2003) described the impact of cancer therapy on not only the female but the male reproductive axis.

Epididymis

After an approximate 72 day process, sperm cells exit the Sertoli cell junction of the seminiferous tubule and enter into the outer structure of the testicles, the epididymis (National Institute of Occupational Safety and Health, 2006). This environment is necessary for spermatozoa to acquire motility and potential fertility. If the sperm are not ejaculated from the epididymis they eventually die and are absorbed by the body.

While controlling their exit from the male reproductive system the epididymis also serves a storage reservoir for spermatozoa. Organized into three distinct regions known as the head (caput), the body (corpus) and the tail (cauda), the epididymal duct is responsible for rhythmic contractions, forcing spermatozoa into the tail. The number of sperm in the distal tail can be altered dramatically by the frequency of ejaculation. Therefore, spermatozoa spending an unusually long time in the epididymal tail may be of poor quality when compared to sperm from males ejaculated routinely, contributing to their lack of viability (Senger, 1999).

In 2006, researchers described a male infertility factor associated with this site (National Institute of Occupational Safety and Health, 2006). The group explained that hazardous chemicals may collect in the epididymis, seminal vesicles, or prostate. These chemicals can kill the sperm, change the way in

which they swim, or attach to the sperm and be carried to the oocyte or unborn child (National Institute of Occupational Safety and Health, 2006).

Further research supported that insufficient sperm delivery could usually be traced back to male infertility issues of the epididymis. Premature or retrograde ejaculation, semen entering the bladder instead of emerging through the penis during orgasm, certain genetic diseases, such as cystic fibrosis, structural problems, such as blockage of the sperm containing epididymis, or damage or injury to the reproductive organs are common examples of male infertility related to compromised sperm delivery (Mayo Clinic, 2014).

The vas deferens of the epididymis are the site of vasectomy procedures in males who want to be unproductive. Medical data has demonstrated that in the cases of men who have previously undergone a vasectomy and desire a return of fertility, can undergo a surgical procedure known as a vasectomy reversal for sperm to be used in assisted reproductive techniques. Similarly, in the cases of men with ejaculatory problems, fertile spermatozoa can be removed from the epididymis and used for artificial insemination (Nagler and Jung, 2009).

Accessory Sex Glands

When a man ejaculates, the mature sperm cells move through the vas deferens, past the seminal vesicles, and the prostate gland (National Institute of Occupational Safety and Health, 1996). During this time the accessory sex glands are responsible for the final altering, packaging, addition of metabolic substrates, and surface coatings for transport of the spermatozoa. Senger (1999) explained that with the help of the epididymis, accessory sex glands produce

secretions which contribute to the liquid, non-cellular portion of semen known as the seminal plasma. Seminal plasma is not required for fertility, but is important in natural insemination where a fluid vehicle for delivery of the sperm is needed.

Male Semen Characteristics

In one of many reviews, Wistuba and colleagues (2007) echoed that spermatogenesis is a highly organized process that requires complex endocrine as well as genomic regulation. This process is supported and mediated by somatic cell types, the Sertoli cells in the tubules and the peritubular myoid cells, and the Leydig cells in the testicular interstitium (Wistuba et al., 2007). During the process of spermatogenesis, the initial cells created are called spermatogonia, following mitosis they become primary spermatocytes that divide meiotically into two secondary spermatocytes. Through Meiosis II each secondary spermatocyte divides into two spermatids that develop into mature spermatozoa, known as sperm cells.

The mammalian sperm cell is composed of a head, a midpiece, and a tail. The head contains the nucleus, the genetic material that contributes the paternal deoxyribonucleic acid (DNA), and is surrounded by the acrosome. Sperm cells come in two types, ‘female’ and ‘male’. Sperm cells that give rise to female (XX) offspring after fertilization differ in that they carry an X-chromosome, while sperm cells that give rise to male (XY) offspring carry a Y-chromosome.

The acrosome contains enzymes that play a key role in the fertilization of the oocyte. Sperm is unable to fertilize an oocyte by natural means if the acrosomal cap and/or the enzymes are not produced. The cap consistently

comes off during the acrosome reaction just prior to fertilization, releasing enzymes that help dissolve the zona pellucida of the oocyte and expose sperm receptors that can then bind the sperm to the oocyte.

Behind the chromosome containing head is a thickened region encompassing the cellular mitochondria called the midpiece. In the case of sperm, the mitochondria are the engines that drive the propeller-like tail to give the sperm its forward motion or motility. The tail flagellates, which propels the sperm cell, at about 1 to 3 mm/minute in humans, by whipping in an elliptical cone movement (Ishijima et al., 1986). In a 2011 study, Sousa and colleagues suggested that one of the differences in sperm fertilization ability between ejaculates may be attributed to the number of sperm in the ejaculate with functioning mitochondria.

For the purposes of this literature review, the characteristics of spermatozoa will be described in common averages, despite the specific variability found among human male samples in the available literature. Research has demonstrated that the mean values for human sperm head dimensions in length are 4.3 μm, width 2.9 μm, area 10.3 μm^2, and perimeter 12.5 μm (Bellastella et al., 2010). These averages are closely related to the 2010 WHO criteria described for normal semen which are as follows; head length 4.1μm, width 2.8 μm, and ratio 1.5 (World Health Organization, 2010).

To claim that there has been controversy over the years in generating a specific normal human spermatozoa reference range would be one of the biggest understatements of the infertility industry. Selecting for normal spermatozoa is

plagued with difficulties, the assessment of 'oval', 'smooth', 'irregular', and 'asymmetric' is extremely subjective (Menkveld, 2010; Auger, 2010). In a 2010 report, Bellastella and collaborators added to the list of researchers who have described that semen samples from different men containing spermatozoa of different sizes.

Researchers proposed that these differences reflect the stresses affecting the spermatozoa, during smearing and air drying of the semen sample that are known to produce swelling of immature sperm heads (Yeung, et al., 1997; Soler et al., 2000), apparent loss of cytoplasmic droplets (Cooper et al., 2004), and cell shrinkage (Katz et al., 1986). The group of researchers explained that the response of the cells to these stresses may be characteristic of each individual male. Under evaluation circumstances, spermatozoa with expanded post-acrosomal regions were also detected in human semen (Ludwig and Frick, 1990). Bellastella et al. (2010) emphasized that if these samples are less mature spermatozoa, detecting them would be of value in diagnosing epididymal dysfunction.

Another factor hindering the evaluation of semen in vitro is that, due to its alkaline nature, a sperm cell does not gain full hypermotility until it reaches the female vagina where the alkaline pH is neutralized by acidic vaginal fluids. This gradual process takes 20 to 30 minutes inside the female reproductive tract. In assisted reproductive technology the challenge has been for researchers to create the same final development environment in vitro.

The sensitivity of the sperm production process is magnified due to the characterization of the sperm cell. The spermatozoon contains a minimum amount of cytoplasm and has the most densely packed DNA known in eukaryotes. Compared to mitotic chromosomes in somatic cells, sperm DNA is at least six fold more highly condensed (Ward and Coffey, 1991). As previously mentioned the production and storage of sperm cells inside the male gonads takes 70 to 74 days from start to finish. Therefore, at any point during this time the testicular assembly line can produce sperm with defects from a number of factors.

Male Semen Evaluation

In 1929, Macomber and Sanders published one of the earliest assessments of sperm concentration in human semen and reported a median of approximately 100 million spermatozoa per milliliter, using blood pipettes and an unidentified counting chamber. In the following decades, systematic studies were undertaken with the examination of semen from men whose partners were interesting discrepancy between results of different centers that surfaced since then has been reviewed by Zukerman et al. (1977) and MacLeod and Wang (1979), especially concerning what should be taken as discriminating values for fertility.

In most normal domestic animals, the evaluation of sperm reveals a generally homogeneous population in individual species. Man, however, is in a small group of species generating semen specimens that exhibit extreme heterogeneity or pleomorphism of sperm morphology between (Menkveld et al.,

1990; Menkveld, 1991; Mortimer, 1994) and even within (Hartmann et al., 1964) specific individuals. Just as the sperm homogeneity observed in animals simplifies the process of determining and defining normality of spermatozoa, the opposite is the case with human spermatozoa. Researchers have unsuccessfully tried to define a ‘fertile’ group or ‘fertile’ individual among human populations (Freund, 1966; Mortimer, 1994). However, inaccurate drawings and increased emphasis on the description of abnormal spermatozoa more than normal spermatozoa (Freund, 1966; Hellinga, 1976; Comhaire et al., 1994) consequently led to an unclear definition of morphologically normal sperm cells with no definitive criteria (Page and Holding, 1951).

In the 1980s, Menkveld (1987) introduced a new concept for the evaluation of sperm morphology, ‘normal spermatozoan’. However, investigators continued to recognize that the morphological data of semen being reported was center-dependent, and highly dependent on the method used to determine the percentage of normal forms, indicating that these differences were procedural (World Health Organization, 1999). This continued to emphasize the need of having clear sperm categorization guidelines applied consistently throughout the industry.

In 1995, Menkveld and Kruger stressed that for a spermatozoon to be considered as morphologically normal by ‘strict’ criteria, the normal biological variations should be kept as small as possible to ensure repeatable evaluation. Thus, the ‘complete’ spermatozoon must be normal as described by the standards of Menkveld (1987, 1991) and Menkveld et al. (1990). This strict

criteria is in contrast with other liberal evaluation systems (Freund, 1966; Eliasson, 1971; Mortimer, 1985; Comhaire et al., 1994) that use lower reference limits to categorize abnormal sperm. The researchers pointed out that in these more generous liberal evaluations, all spermatozoa that are not classified as abnormal will be regarded as normal, resulting in two sperm populations (Menkveld and Kruger, 1995). Therefore, just because a sperm is not abnormal, considering it normal may lead to a faulty classification of the fertility potential of a specific male.

In the same study, Menkveld and Kruger (1995) described the characteristics that a sperm cell must exhibit to be considered morphologically normal by strict criteria. The sperm head must have a smooth oval configuration with a well-defined acrosome comprising 40.0 to 70.0% of the anterior sperm head. Normal head dimensions are head length and width between 3.0 to 5.0 µm and 2.0 to 3.0 µm, respectively, as suggested by Eliasson (1971). No neck, midpiece, and/or tail defects must be present. The midpiece must be slender, axially attached, ≤ 1µm in width, and approximately 1.5 times the head length (Menkveld and Kruger, 1995). Tails must be straight, uniform, slightly thinner than the midpiece, uncoiled, and ± 45 µm long (Menkveld and Kruger, 1995).

In their report, Menkveld and Kruger (1995) reaffirmed that most researchers are in agreement when describing morphological evaluation of human spermatozoa as one of the most controversial semen parameters. The importance of morphology is seen in terms of its role in establishing male fertility potential, and its role as a prognostic parameter for fertilizing ability in vivo

(Menkveld et al., 1990) or in assisted reproduction (Fraser and DasGupta, 1993; Kruger, 1994). Adding to the controversy, sperm reference ranges continue to change from decade to decade and vary from one publication to another.

Therefore, although semen analysis is routinely used to evaluate the male partner in infertile couples, sperm measurements that discriminate between fertile and infertile men are not well defined (Guzick et al., 2001). A typical semen analysis is used to grade the quality of a sperm sample; the number of sperm per milliliter of ejaculate, as well as the morphology and motility of the sperm are measured. Common morphological defects observed are double heads, double tails, abnormally sized acrosomes, missing acrosomes, kinked tails, missing heads, missing acrosomes, short tails, and abnormally sized heads.

Currently the majority of sperm samples are graded under one of two grading criteria: Kruger's Strict criteria, as described above, or The World Health Organization criteria. In a 2010 report titled, 'World Health Organization reference values for human semen characteristics', Cooper et al. described the updated WHO guidelines. The following lower one-sided reference limits, with 95.0% confidence, were generated from men whose partners had been trying to get pregnant less than or equal to 12 months: semen volume, 1.5 ml; total sperm number, 39 million per ejaculate; sperm concentration, 15 million per ml; vitality, 58.0% live; progressive motility, 32.0%; total motility, 40.0%; and morphologically normal forms, 4.0% (Cooper et al., 2010).

Research has shown that a number of factors may influence the accuracy of a semen analysis results; in addition results for a single man can have a large

amount of natural variation over time. Advanced research has clarified that male age and reproductive threats can contribute to the malformation of sperm cells, hindering the capability of fertilization, and male reproduction (Moline et al., 2000). For this reason, Weschler (2002) had previously suggested that a subfertile result must be confirmed with at least two further analyses.

As couples wait longer to have children, it is important to acknowledge that paternal factors provide an equal emphasis on reproductive success. Essentially, it is just as important to understand the characteristics of the semen in these infertile men and how to correctly distinguish and treat them for increased fertility.

Human Infertility

Infertility is an increasing public health issue in the United States that affects women, men, and couples. In a 2014 national report, the Centers for Disease Control and Prevention (CDC) explained that, depending upon the underlying cause, infertility can be treated by gynecologists, urologists, and reproductive endocrinologists using a range of medical options, including advice on the timing of intercourse, drugs to stimulate ovulation, surgery, and ART procedures (Centers for Disease Control and Prevention, 2014).

In the past two decades, there has been an explosion of interest in the scientific advancement of human reproduction and assisted conception. It has been theorized that the increase in assisted reproductive technology rates is due to the present societal trend of parents delaying childbirth. There is an ongoing debate among researchers over the fact that human fertility is actually declining

or that fertility treatments are increasing because couples are waiting longer to conceive, needing more assistance at an advanced age.

As opposed to our parents' generation, a majority of individuals today are focused more on their careers in their 20's and 30's, while waiting to start families later in life. As a result men and women are attempting to conceive at an older age with increased years of possible exposure. For couples who do end up experiencing infertility, a collective progression of medical treatments is available through assisted reproductive technology and research continues to evolve daily.

For a human pregnancy to occur, every part of the complex reproductive process has to take place at just the right time. Females release at least one mature oocyte from one or both ovaries to be picked up by the fallopian tube. Males produce mature, viable spermatozoa that swim up the female cervix, through the uterus and into the fallopian tube to fertilize the newly released oocyte(s). The fertilized oocyte then travels down the fallopian tube to the uterus, where it implants and grows into a fetus. History has shown that a number of known and unknown factors can disrupt this process at any step.

Infertility is defined by the World Health Organization (2014) as a disease of the reproductive system characterized by the failure to achieve a clinical pregnancy after 12 months or more of regular, unprotected sexual intercourse, excluding reasons such as breastfeeding or postpartum amenorrhea. In the U.S., a commonly used definition of infertility is when a woman under 35 has not conceived after 12 months of contraceptive-free sexual intercourse and a woman

over 35 has not conceived after 6 months of contraceptive-free sexual intercourse (Cooper et al., 2010).

The reasons for infertility can involve one or both partners and can be congenital and present from birth and/or from environmental or lifestyle factors. In some instances, a cause for infertility is never found and it is possible that a combination of several minor factors in both partners underlie these unexplained fertility problems. Therefore, the more knowledge we gain on the factors that affect human fertility, the more tools we will have to decipher the problem.

Prior studies have shown a strong paternal effect of sperm DNA damage on in vitro fertilization outcome, including reduced fertilization, reduced embryo quality and cleavage rates, reduced numbers of embryos developing into blastocysts, increased percentage of embryos undergoing developmental arrest, and reduced implantation and pregnancy rates (Simon et al., 2014). The quality of the semen sample is also responsible for the advancement of certain maternal gestational factors, such as the development of the placenta. In addition, recent research has shown that damaged or aged sperm possibly poses later health risks for the offspring of older fathers.

Understanding male sperm production is important in appreciating the vulnerability of the sperm to environmental and chemical exposures. One common misunderstanding is that the male manufactures millions of sperm daily, therefore, activities engaged in weeks or months earlier do not have an effect upon the sperm quality. Although the human male does produce millions of sperm daily, it takes approximately 72 days to actually create and store the

sperm within the testicles. Therefore, there is over two months of time before conception that the male can be exposed to environmental circumstances that could slow or harm the healthy genetic development of the sperm cell (Pressinger, 1997). In addition, as the reproductive age of the human male increases, researchers have become less confident in the well accepted theory of an infinite male fertility period.

Closer evaluation has suggested a number of hypotheses as to why male reproductive senescence occurs. For example, some researchers have speculated that programed gene expression changes are responsible. While others have proposed it is due to cumulative damages caused by biological processes. However, whether senescence as a biological process can be slowed down, halted, or even reversed; is a subject of current speculation and research. Rather than becoming aged, as the term cellular senescence suggests, it is hypothesized that these specific sperm cells are representative of a change in cell state.

Unlike virtually every other cell in the body, sperm cells have no defense mechanism. Any toxin that damages a sperm cell causes it to generate high levels of free radicals that can damage surrounding cells as well. Determining the factors that lead to increased semen damage will help to initiate more effective treatment plans that may include: taking supplemental antioxidants, improved healthier lifestyle, varicocele repair, medication revaluation, and avoidance of various types of heat and chemical exposure.

Recommendations already include suggestions for males to avoid exposure to some common work and environmental toxins like: organic solvents, oil products, processed foods, chlorinated and fluorinated water, paint, photographic supplies, irradiation, heat, combustion engine exhaust fumes, and heavy metals. The past 10 years have shown an increase in studies linking weak or defective sperm to employment in occupations with exposure to chemicals and pesticides (Strohmer et al., 1993).

Dependent upon patient diagnosis, couples may start fertility treatment with partially assisted reproductive techniques and progress to more advanced methods as treatment progresses. One of the first methods commonly implemented is ovarian stimulation, a hormonally controlled procedure in which females receive drug treatments to induce ovulation through the production of multiple follicles. At the time of ovulation, either sexual intercourse is performed by the couple or the use of additional assisted reproductive techniques, such as artificial insemination, are implemented. AI can be performed through intrauterine insemination (IUI) of the sperm into the female reproductive tract using artificial means other than sexual intercourse.

The next level of treatment is the most frequently used procedure in assisted reproductive technology. In vitro fertilization, or IVF, is the joining of sperm and oocytes outside of the body in production of a fertilized zygote. Following three to five days of in vitro culture, the embryo(s) are transferred into the female reproductive tract. Embryo transfer (ET) is performed on a

corresponding date with the cyclic female uterus by using artificial means to implant the fertilized embryo.

In some instances, a further assisted reproductive technique may be implemented. Intracytoplasmic sperm injection (ICSI), most commonly used for male infertility problems, is an in vitro fertilization procedure that occurs outside of the body where micromanipulation of a single sperm cell is injected into the oocyte. As with IVF, the developing embryo is transferred through artificial means into the female reproductive tract after three to five days of development.

The Use of Assisted Reproductive Technology

The Society for Assisted Reproductive Technology (SART) reported that in 2012, of the group's 379 member clinics in the United States, 165,172 assisted reproductive cycles were performed (Society of Assisted Reproductive Technology, 2013). These procedures resulted in the birth of 61,740 babies, an increase of more than 2000 infants from 2011. Although the use of ART is still relatively rare as compared to the potential demand, its use has double over the past decade. In 2012, the American Society of Reproductive Medicine reported an estimated 3.9 million babies born in the U.S., the number of IVF babies constituting over 1.5% of all births in the U.S. (American Society of Reproductive Medicine, 2014). This was the largest number of cycles, of babies and percentage of babies born through IVF ever reported (American Society of Reproductive Medicine, 2014).

Numerous previous analyses have shown that women in the United States who make use of medical help for fertility problems are a highly selective group

among those who have fertility problems. Data from nationally representative surveys, primarily the National Survey of Family Growth, but also clinic-based studies, have shown that fertility-impaired women who use infertility services are significantly more likely to be married, non-Hispanic white, older, more highly educated and more affluent than nonusers (Chandra and Stephen, 2008; Greil et al., 2011; Hirsch and Mosher, 1987; Kalmuss, 1987; Nachtigall, 2006; Staniec and Webb, 2007; Stephen and Chandra, 2000; Wilcox and Mosher, 1993).

Reasons for the disparities in use of infertility services may include access barriers such as the significant cost of medical services for infertility and the lack of adequate health insurance to afford the necessary diagnostic or treatment services (William, 1997; Smith et al., 2011). Unlike the extensive infertility healthcare in other countries, such as Denmark, currently only 15 U.S. states have passed insurance mandates to cover ART. Unfortunately, there is evidence to suggest that these mandates have done nothing to better the difference in rates of infertility treatment by race or ethnicity and socioeconomic status (Bitler, 2012).

After a 1995 review, Schmidt and colleagues assumed that about 50.0% of all Danish couples experiencing infertility seek ART treatment. In Denmark, the number of initiated treatments with IVF and ICSI performed at public and private fertility clinics has increased by 83.0%, from approximately 6,000 per year to more than 11,000 per year, within the last 10 years (Schmidt, 2006; The Danish Fertility Society, 2009). However, infertility treatment is widely available in Denmark within both the public and private healthcare systems (Nyboe Anderson

et al., 2005). The public system has offered all infertile couples up to three in vitro fertilization treatments free of charge.

However, Aitken (2014) explained that ART treatments are often delivered without critically considering the underlying causes of the condition or seriously contemplating the long-term consequences of the current enthusiasm for such therapy. Critical factors supporting the need of couples to engage in ART can range from advanced maternal age to a variety of lifestyle factors, such as smoking and obesity, which are known to compromise the developmental potential of the oocyte and DNA integrity in the spermatozoa.

Advanced Age and Human Infertility

As the societal trend for older parents to have children increases, health concern about age-associated risks of infertility, abnormal pregnancies, and birth defects remain a top concern. In a 2008 publication, Maheshwari claimed that since the 1980's infertility rates in humans have increased by 4.0%, mostly from problems with fecundity due to an increase in age.

Conversely, in a national survey conducted from 1982 to 2010, Chandra and colleagues (2013) reported that infertility rates have actually decreased among U.S. women of childbearing age from 8.5 to 6.0%. One explanation of this common contradiction may arise from data that showed an increase in actual fertility treatments. It is quite possible that although fertility 'treatments' have increased because many couples are having children later in life, it is debatable that infertility 'rates' among humans are increasing as well.

Presently, the most common difference in fertility being observed among couples is age. Research shows that individuals need more help in their 40's to obtain pregnancy than in their 20's. The reasons behind this increased uptake in ART treatments are complex. Aitken (2014) proposed that this was a consequence of the high incidence of spontaneous male infertility and the advanced age at which couples are now attempting to start their families. His 2014 research demonstrated that age has a dramatic effect on the human capacity to reproduce (Aitken, 2014).

A growing body of literature has been compiled on the influence of maternal age on adverse fetal birth outcomes (Abel et al., 2002; Astolfi and Zonta, 1999; Croen et al., 2007; de la Rochenbrochard and Thonneau, 2002; Salihu et al., 2003; Salihu et al., 2008). Such studies have produced a wealth of evidence of an association between advanced maternal age and increased risks of fetal loss, preterm delivery, and small size for gestational age (Astolfi et al., 2006; Astolfi and Zonta, 1999; Fretts and Usher, 1997; Nahum and Stanislaw, 2002; Raymond et al., 1994; Salihu et al., 2008). Both in general and in relation with specific pathologies, researchers have shown that female age induced an overall reduction in the chance of bearing a child, and in particular a healthy child (Cnattingius et al., 1992; Fretts et al. 1995; Bianco et al., 1996; Dollberg et al., 1996; Breart, 1997; Faden et al., 1997; Horta et al., 1997; Tarin et al., 1998; Gilbert et al., 1999; Pattenden et al., 1999; Astolfi and Zonta, 2002).

The pressure of the modern career-minded women to meet their twin goals of having a family and achieving their professional aspirations makes

delaying child bearing inevitable (Aitkin, 2014). This trend is observed in the number of first births to US women aged 35 to 39 years that increased by 36.0% between 1991 and 2001 and the rate among women aged 40 to 41 increased by a staggering 70.0% (Heffner, 2004).

Lansac (1995) demonstrated that female fecundity declines precipitously by the fourth decade of life due to oocyte loss, increased risks of miscarriage, trisomies, and/or chromosomal defective offspring. A decade later, Aitken (2014) likewise supported the idea that female fertility declines precipitously between the ages of 35 and 42 years. Although, coming from both historical records and data generated by assisted conception clinics, the Human Fertilization and Embryology Authority (2010) claimed the decline can be seen as controversial.

Studies that have examined paternal age as a risk factor for adverse birth outcomes have yielded mixed results as well. Although some studies found an association between advanced paternal age and increased risk of spontaneous abortion (de la Rochenbrochard and Thonneau, 2002; Kleinhaus et al., 2006; Slama et al., 2005), preeclampsia (Harlap et al., 2002), stillbirth (Nyboe Anderson et al., 2004; de la Rochenbrochard and Thonneau, 2002), schizophrenia (Kühnert and Nieschlag, 2004; Malaspina et al., 2001), autism (Reichenberg et al., 2006), low birth weight (Tough et al., 2003), and other birth defects (Kühnert and Nieschlag, 2004; Savitz et al., 1991; Yang et al., 2007), other studies found no evidence of a relationship between advanced paternal age and adverse fetal birth outcomes (Abel et al., 2002; Chen et al., 2008; Nahum and Stanislaw, 2003; Parker and Schoendorf, 1992).

This midlife decline in human fertility sets our species apart from all other primates, where mortality and reproductive lifespan are coincident and very few individuals experience reproductive senescence before death (Alberts et al., 2013). The reason for this is unknown but may simply be that we have, as a consequence of improvements in primary health care, managed to push the limits of human mortality beyond the lifespan of the primordial follicle population (Aiken, 2014) or the age associated ability to produce quality spermatozoa (Singh et al., 2003; Schmid et al., 2007; Das et al., 2013).

In a 2007 study, Yang et al. explained that although the association between maternal age and the risks of birth defects has been well studied, the role of paternal age has received relatively little attention. As early as 1912, Weinberg hypothesized a genetic component in the effect of advanced age suggesting that sporadic cases of achondroplasia, a genetic disorder, could be associated with paternal ageing. However, it was not until the past decade that research has become more heavily focused on male age as a factor in human infertility. In 2013, Chandra and Wu acknowledged that among men, some form of infertility was reported by 9.4% of those aged 15 to 44 and by 12.0% of those aged 25 to 45 from 2006 to 2010.

The current societal trend for older parents to have children has raised public health concern and encouraged more research to be designed on male age-associated risks of abnormal pregnancies and birth defects. Although spermatogenesis continues well into male senescence and some men of advancing age can father children, in two separate studies, Kidd and colleagues

(2001) and Slotter and colleagues (2004) both engaged the subject of male fecundity declining with age. It is well known that men have been able to father children well in to their 90's, therefore it seems difficult to contrast the loss of fertility due to advanced age in men versus women (Kidd et al., 2001; Slotter et al., 2004). However, the risks of abnormal pregnancies and heritable effects associated with advancing paternal age are poorly understood, thus increasing the development of interest in exploring this outcome.

Research has demonstrated that older men produced more sperm with DNA damage as a consequence of age-associated increased oxidative stress in their reproductive tracts (Barnes et al., 1998; Barroso et al., 2000). In 1997, Kodama and colleagues reported an association between oxidative DNA damage in sperm and male infertility.

Studies that followed showed that as men age the quality of their gametes deteriorates (Singh et al., 2003; Schmid et al., 2007; Das et al., 2013). As a result, the spermatozoa of ageing males contain much more DNA damage than their younger counterparts. Studies on the Brown Norway rat and the senescence accelerated mouse (SAM) both suggested that the origin of this age-dependent increase in DNA damage in the germ line is oxidative, reflecting the general relationship between oxidative stress and ageing observed in most biological systems (Paul et al., 2011; Smith et al., 2013).

Alternately, apoptotic functions of spermatogenesis may be less effective in older males resulting in the release of more sperm with DNA damage (Brinkworth et al., 1997; Print and Loveland, 2000). Brinkworth and Schmid

(2003) reported that the testes of older male mice have lower apoptotic frequencies than young adults. It was shown that oxidative stress significantly increased the frequencies of apoptotic spermatocytes in young male mice while reducing testicular apoptosis in older males (Barnes et al., 1998).

In 2002, Morris and colleagues reported that sperm DNA damage was positively correlated with donor age and with impairment of post-fertilization embryo cleavage following ICSI, indicating an overall decline in the integrity of sperm DNA in older men. Aitken and colleagues (2003) explained that oxidative stress can damage sperm DNA, as well as mitochondrial and nuclear membranes. Consistent with the hypothesis of the importance of oxidative damage to sperm, it was reported that high antioxidant intake was associated with better semen quality, especially motility within the same study group (Eskenazi et al., 2005).

In 2007, Schmid and colleagues found associations between male age and sperm DNA strand damage in a non-clinical sample of active healthy non-smoking workers and retirees. Sperm of older men had significantly higher frequencies of sperm with DNA damage measured under alkaline conditions, which is thought to represent alkali-labile DNA sites and single-strand DNA breaks (Schmid et al., 2007). At the conclusion of the study, Schmid and colleagues (2007) determined that age-related increases in sperm DNA damage predict that men who delay fatherhood may have increased risks of unsuccessful and abnormal pregnancies as a consequence of fertilization with damaged sperm.

Research has also demonstrated that increased sperm DNA damage has been associated with chromosomal abnormalities, developmental loss and birth defects in mouse model systems (Marchetti et al., 1997; Haines et al., 1998; Hughes et al., 1999; Marchetti et al., 2004), and with increases in the percentage of human embryos that fail to develop after ICSI (Morris et al., 2002). Previous studies have explained that each successive fragmentation introduces a slight risk of error in the genetic material of the new sperm, and this is then passed on to the child (Wyrobek et al., 2006).

Previous studies have also demonstrated that as the age of the father increased, the risk of miscarriage and, if the pregnancy does carry to term, disease in the offspring, increased in parallel (Aitken and Krausz, 2001; Aitken et al., 2004; Kleinhaus et al., 2006). There is a sum of epidemiological evidence that have suggested the incidence of abnormal reproductive outcomes and heritable defects increase with paternal age (Tarin et al., 1998; de la Rochebrochard and Thonneau, 2002), including pregnancy loss (Risch et al., 1987; de la Rochebrochard and Thonneau, 2002), developmental and morphological birth defects (Lian et al., 1986), gene mutations (Crow, 2000; Tiemann-Boege et al., 2002), various aneuploidy and chromosomal syndromes (Slotter et al., 2004), and diseases of complex aetiology, such as prostate cancer (Zhang et al., 1999).

Research published from the Columbia University School of Public Health in 2006 suggested that women who become pregnant by older men are at far greater risk of having a miscarriage (Kleinhaus et al., 2006). The researchers

noted that the risk of miscarriage appeared to rise along with the father's age, regardless of the mother's age. Even after a range of other risk factors which contribute to miscarriage were taken into account, such as smoking during pregnancy and maternal diabetes, the risk was still higher (Kleinhaus et al., 2006).

This study analyzed data from a survey of nearly 14,000 pregnant women undertaken in Jerusalem between 1964 and 1976 (Kleinhaus et al., 2006). The group also demonstrated that the risk of losing the pregnancy was 60.0% higher when the father was 40 or older, compared to when he was 25 to 29 years old. The risk of losing the pregnancy was approximately three times greater when the man was between 35 and 39 years of age, than if he were younger than 25 (Kleinhaus et al., 2006).

Advanced age has been a significantly studied factor in the fertility of human females, and more recently additional attention has been focused males. Evidence has suggested that men may in fact have a biological time clock slightly similar to that of women. However, men seem to have a gradual rather than abrupt change in fertility and the potential ability to produce viable offspring.

Aside from age, there are a number of other possible factor(s) in humans that play a role in the infertility of a couple. However, it is extremely important to note, that research has proven about 40.0% of the issues involved with infertility are due to the man, another 40.0% are due to the woman, and 20.0% result from complications with both partners (Hudson, 1987).

Environmental Lifestyle Factors and Male Infertility

In a 2014 research article that evaluated lifestyle and male fertility, Jurewicz and colleagues explained that semen quality in the adult male can be affected by a number of environmental and lifestyle factors. The group explained that the increasing trend in male infertility observed in recent years may be associated at least in part with these factors, which are compounded by a change in lifestyle. Lifestyle associated exposures including cigarette smoke, alcohol, caffeine, use of mobile phones, and body mass index (BMI) have been studied in relation to male semen quality (Fejes et al., 2005; Jensen et al., 2010; Magnusdottir et al., 2005; Ramlau-Hansen et al., 2007; Li et al., 2009).

Additional research demonstrated that between 10.0% and 15.0% of all couples experience fertility problems due to a variety of causes (Schmidt et al., 1995; Eugster and Vingerhoets, 1999; Juul et al., 1999), and infertility is increasing in the industrialized countries, possibly due to social and behavioral factors along with environmental exposures (Skakkebaek et al., 2006). Present research has shown that external factors linked to lifestyle negatively affect spermatogenesis, both at the central and gonadal levels (Rato et al., 2014).

It has been shown that epidemiological and controlled animal studies in the lab suggested that paternal nutritional and toxicological exposures, as well as age, impact the health of the male and the health of his children. These studies suggested a potential trans-generational impact of paternal effects (Curley et al., 2011).

Male exposures, in isolation from female exposures, have been shown in experimental studies to be capable of affecting the entire spectrum of the reproductive health endpoint (Olshan and Faustman, 1993) through mechanisms involving sperm. Such effects most likely occurred from male exposures in the three months prior to conception (Schrader and Kesner, 1993). This suggestion parallels the human spermatogenesis timeframe, approximately 72 to 74 days, including the transport of sperm through the ductal system.

An important lifestyle-dependent factor that adversely affects spermatogenesis is obesity (Jurewicz et al., 2014). Several studies have shown up to a threefold higher incidence of obesity in infertile men than in those with normal semen quality (Hammoud et al., 2008a; Magnusdottir et al., 2005).

Also, studies on caffeine intake and semen quality have shown contradictory results. Some researchers suggested no associations (Oldereid et al., 1992; Ramlau-Hansen, 2008), whereas others found reduced sperm concentration as well as reduced total sperm count and motility (Jensen et al., 2010; Sobreiro et al., 2005). Several studies also examined the effect of smoking and alcohol drinking on sperm parameters, but their results were inconsistent (Jurewicz et al., 2014). Additional studies (Marinelli et al., 2004; Povey et al., 2012) but not all (Li et al., 2011; Ramlau-Hansen et al., 2007; Vine, 1996) suggested that smoking and alcohol had a limited effect on semen quality. Other studies have shown factors such as smoking, alcohol and caffeine consumption to be associated with increased genetic damage in blood cells (Park and Kang,

2004; Glei et al., 2005, Wyrobek et al., 2005a), but little is known about their effects on genetic damage in sperm (Wyrobek et al., 2005b).

Nevertheless, growing reviews of male subfertility have highlighted how aspects of male lifestyle may significantly increase the risk of subfertility (Li et al., 2011; Sadeu et al., 2010). These reviews further suggested that higher age, smoking, alcohol consumption, and psychological stress were risk factors for poor semen quality (Li et al., 2011; Sadeu et al., 2010).

BMI and Male Infertility

The World Health Organization (2014) defines obesity as a BMI ≥ 30 kg/m^2. The Journal of the American Medical Association reported that obesity is a public health disorder that affects more than 34.9%, 78.6 million, of U.S. adults (Ogden et al., 2014). In addition, infertility is a public health disorder that affects 10.0% of the worldwide population (Monmandi et al., 2013). Despite one third of infertility cases being attributed to male factors, studies on the impact of BMI on male fertility are still very limited and controversial as compared to the multiple studies evaluating the impact of overweight in women's fertility (Monmandi et al., 2013).

Research has demonstrated increased evidence that female obesity has a negative effect on assisted reproductive technology outcomes (Bellver et al., 2010; Luke et al., 2011; Pasquali et al., 2003). Excessive weight in women undergoing ART treatments has been associated with lower pregnancy rates, lower live birth rates, fewer normally fertilized oocytes and the need for higher doses of gonadotropins (Bellver et al., 2010; Luke et al., 2011; Maheshwari et al.,

2007; Shah et al., 2011). Notably, recent studies have demonstrated the effects of overweight and obesity on reproductive health in which both members, male and female, are at an increased risk of subfertility (Ramlau-Hansen et al., 2007).

As obesity has become a more serious health problem in the western world, researchers speculated that it is partly to blame for the decline in male fertility. The average U.S. male has a BMI of 29, which is highly overweight (Centers for Disease Control and Prevention, 2012). Some investigators agreed that high BMI levels may reduce male fertility and associated it with reduced semen quality and hormone alterations (Jensen et al., 2004; Kort et al., 2006; Fejes et al., 2005; Fejes et al., 2006). In addition, overweight men may be at greater risk of erectile dysfunction (Fung et al., 2004), which could lead to reduced fertility.

In 2008, researchers found a higher incidence of oligozoospermiea and a greater prevalence of low progressive sperm count in male patients with increased BMI levels (Hammoud et al., 2008b). Additional research demonstrated that overweight and obesity in males have been associated with poorer semen quality (Sermondade et al., 2012), higher sperm DNA damage (Chavarro et al., 2010; Kort et al., 2006; Tanrikut et al., 2010), and infertility (Sallmén et al., 2006). In 2011, from a sample of 2,035 male patients, Shayeb and colleagues reported that obese men were more likely to have lower semen volume and fewer morphologically normal spermatozoa than men with normal BMI.

Determined through data obtained from a large patient sample size, Belloc and colleagues (2014) reported that semen volume decreased from 3.3±1.6 to 2.7±1.6 mL when BMI increased from normal, 20 to 25 kg/m^2, to extreme male obesity, >40 kg/m^2, respectively. In addition, the group reported decreased semen concentration from 56.4±54.9 to 39.4±51.0 million/mL, total sperm count from 171±170 to 92±95 million, and progressive motility from 36.9±16.8 to 34.7±17.1% when male BMI increased from normal to extreme obesity (Belloc et al., 2014). The percentage of cases with azoospermia and cryptospermia also significantly increased in connection to higher BMI levels (Belloc et al., 2014). However, morphology was not affected as reported by the group.

As BMI becomes an increasingly debated topic among male fertility, more than one cause of its relationship with couples' reproductive success is being investigated. Some research has shown that obesity has been associated with significant disturbance in the hormonal environment that can affect the reproductive system.

In 2007, Nguyen and collaborators demonstrated that excess weight may be linked with altered testosterone, estradiol levels, poor semen quality, and infertility (Nguyen et al., 2007). When research participants were divided into eight categories of male BMI patients, a trend of increased male infertility and increased male BMI was observed (Nguyen et al., 2007). Nguyen and colleagues (2007) explained that more research is needed to see if weight loss improves fertility for men with high BMI levels.

Additional research indicated that male BMI is inversely related to androgens levels and positively related to estrogens (E_2) levels resulting in a hormonal profile consistent with hypogondotropic hyperestrogenic hypoandrogenemia (Hammoud et al., 2008a; Giagulli et al., 1994; Chavarro et al., 2010). The higher E_2 levels are reported to have a deleterious effect on endogenous gonadotropin secretion as they interfere with GnRH pulsatility (Hammoud et al., 2008a; Akingbemi, 2005).

The specific relationship between male BMI and ART outcomes have been examined even less extensively. Due to the scarce and controversial literature (Keltz et al., 2010; Colaci et al., 2012) available on this topic, it is difficult to correctly assess the origins of differences between these research studies. These discrepancies continue to support the importance of further evaluating the relationship between male obesity and ART outcomes.

In 2010, Keltz and colleagues conducted a retrospective analysis that showed that couples with an overweight or obese male partner, BMI >= 25 kg/m^2, undergoing traditional IVF had lower clinical pregnancy rates than couples with a lean male. However, they did not find this same association in ICSI cycles (Keltz et al., 2010).

In 2012, Colaci and co-workers claimed to initiate the first prospective study that addressed the relationship between male BMI and ART outcomes in which these associations were adjusted for the most important female characteristics that are known to have a critical effect on the overall outcome (Weghofer et al., 2005; Chung et al., 2003; Omland et al., 2005; Dunson et al.,

2004). The group actually reported higher fertilization rates among obese men than among normal weight men in conventional IVF cycles and found no significant associations between male BMI and the proportion of poor quality day-3 embryos, slow embryo cleavage rate, or accelerated embryo cleavage rate (Colaci et al., 2012).

The findings of Colaci and colleagues (2012) were in agreement with Bakos and colleagues (2011) who reported no association of male BMI with overall fertilization rate or day-3 in vitro embryo quality. However, in their 2011 study, Bakos et al. found a significant reduction of blastocyst development and lower pregnancy rate associated with increasing male BMI. In support of those results, a recent animal study concluded that male obesity was related to reduced embryo cleavage, decreased development to the stage of blastocyst, lower implantation rate, and lower fetal development (Mitchell et al., 2011).

Samavant and colleagues performed a preliminary study in 2014 and demonstrated that the acrosome reaction in sperm is impaired in obese men. The study showed a reduced response to progesterone and an elevated spontaneous acrosome reaction (Sp-AR), associated with altered circulating levels of E_2 and sperm cholesterol content in males with higher BMI levels (Samavant et al., 2014).

In addition, practitioners have contested that as BMI increased the DNA fragmentation rate of sperm increased as well, creating a dramatic reduction of sperm quality (Kort et al., 2006; Chavarro et al., 2010; Fariello et al., 2012; La Vignera et al., 2012). As previously mentioned the more sperm with fragmented

DNA, the higher the chances of miscarriage and lower the chances of conception (Sakkas and Alvarez, 2010).

Adding to the controversy, in 2015 Schliep and colleagues reported that weight status did not influence fecundity among couples undergoing infertility treatment. However, the group stressed that given the limited and conflicting research on BMI and pregnancy success among IVF couples, further research designed to include other adiposity measures is needed (Schliep et al., 2015). Although the influence of male BMI on fertility remains controversial and understudied, there does seem to be some multifactorial relationship, therefore, additional studies are needed to determine the association.

Male Environmental Exposures

Caffeine

Studies on caffeine intake and semen quality have shown contradictory results as well; some suggested no associations (Oldereid et al., 1992; Ramlau-Hansen, 2008). Others found caffeine exposure reduced sperm concentration, total sperm count, and sperm motility (Jensen et al., 2010; Sobreiro et al., 2005; Vine, 1996). In their 1999 study, Sarkaria and colleagues demonstrated that caffeine is an efficient inhibitor of DNA double-strand repair, which may explain the increased double-strand DNA damage in sperm after high-dose caffeine consumption.

In 2007, Schmid and colleagues found that men with caffeine consumption of about three cups per day had significantly higher frequencies of sperm with DNA damage as measured under neutral, but not alkaline conditions compared

to men with less caffeine consumption. Whereas, in 2014 Jurewicz et al. reported that drinking coffee one to six times per week was related to an increase in the percentage of motile sperm but also in sperm head abnormalities. The group additionally associated drinking coffee every day with an increase in sperm neck abnormalities (Jurewicz et al., 2014). However, it was noted that the estimation of caffeine intake based on self-report can be a problem because cups of coffee vary in strength related to brewing and brand and caffeine is present in many products that would not necessarily be recognized and reported during the interview (Jurewicz et al., 2014).

Chemical Exposure

As early as 1972, it was shown that paternal exposure to mutagenic compounds increased the rate of spontaneous abortions in animals (Epstein et al., 1972). However, in humans this relationship remains relatively unclear. For vinyl chloride (Infante et al., 1976), anesthetic gases (Tomlin, 1979), dibromochloropropane (Kharrazi et al., 1980), chloroprene (Sanotsky, 1976), smelter work (Beckman and Nordström, 1982), waste water exposures (Morgan et al., 1984), and organic solvents (Taskinen et al., 1989), effects on human fertility have been suggested, but the data either have been contradictory or remain unconfirmed.

In 1983, Donner et al. reported that rubber chemicals contained several microbial mutagens and Lindbohm and colleagues (1983) reported an increased risk of abortion observed among women exposed to rubber chemicals. An excessive rate of spontaneous abortion was also found among the wives of

workers in a waste water treatment plant of a petroleum refinery (Morgan et al., 1984) and among the wives of workers exposed to organic solvents (Taskinen et al., 1989).

Researchers also found an association with male infertility among some paternal occupations: metal-plate and constructional steel workers, crushers and grinders, sewage, workers caring for fur-bearing animals (Lindbohm et al., 1984), and mechanics and repairers of motor vehicles (McDonald et al., 1989). In a 1991 study, Lindbohm and colleagues evaluated 25 specific mutagens or groups of mutagens. Paternal exposure to ethylene oxide, rubber chemicals, solvents used in petroleum refineries, and solvents used in manufacture of rubber products were the only four chemicals that the group found to be associated with an increased risk of spontaneous abortion. However, Lindbohm and colleagues (1991) were unable to separate the routes of exposure; harmful substances transmitted to the pregnant woman by contact with clothes or by semen leading to secondary maternal exposure.

Among the chemicals, Lindbohm and colleagues (1991) acknowledged that ethylene oxide had been identified as a mutagen by almost all mutation assays, including the dominant lethal assay (International Agency for Research on Cancer, 1988). Exposure to this chemical has also been associated with spontaneous abortion in women who use ethylene oxide to sterilize hospital instruments (Hemminki et al., 1982).

Several studies have addressed the pesticide dibromochloropropane as a proven cause of male infertility. A report by Kharrazi et al. (1980) suggested a

threefold increased risk of miscarriage in the offspring of exposed males. Additional studies indicated a decrease in the proportion of male offspring after paternal exposure (Goldsmith et al., 1984; Potashnik et al., 1984). Dibromochloropropane exerts its effects through direct testicular toxicity, which is not known to occur from other more commonly used pesticides (Kharrazi et al., 1980).

In 1993, Moses described studies of both maternal and paternal pesticide exposure in relation to such endpoints as infertility, miscarriage, stillbirth, preterm delivery, low birth weight, and birth defects. For miscarriage, Olshan and Faustman (1993) published clear experimental evidence of a paternal effect. In addition, epidemiologic literature offered at least some replicated indications of an environmental contribution to human infertility (Savitz et al., 1994).

A 1996 report from the National Institute for Occupational Safety and Health (NIOSH) identified a number of workplace substances such as, lead and radiation as reproductive hazards for men. The study explained that the harmful substances can enter the body by inhalation, contact with the skin, or ingestion, if workers do not properly wash their hands before eating, drinking or smoking.

In 1997, Savitz et al. claimed that despite the generally favorable health experience of farmers, potential adverse reproductive health effects associated with pesticides were of concern. The group identified five activities that were presumed to involve direct pesticide exposure: mixing or applying crop herbicides, crop insecticides and fungicides, livestock chemicals, yard herbicides, and building pesticides (Savitz et al., 1997). The results of the study provided

some indication that male farm activities may influence the risk of preterm delivery, particularly when occurring in combination with reported applications of specific chemicals on the farm (Savitz et al., 1997).

The overall implication of the study results of Savitz and colleagues (1997) added to the interest in a possible role of male pesticide exposure in adverse pregnancy outcome and directed attention to both preterm delivery and miscarriage. With their refined measures of exposure and outcome, the researchers pointed out that detailed consideration of male pesticide exposure in relation to sperm function and genetic alterations would help to bridge experimental and epidemiologic studies (Savitz et al., 1997).

The blood-testis barrier is a defense mechanism that has been shown to protect testicular cells from direct exposures to high levels of hazardous chemicals in the blood (Cheng and Mruk, 2012). Vigeh et al. (2011) supported this theory with their review of lead toxicity on reproductive hormones. The group suggested that lead's main influence on male reproduction probably occurred by altering the reproductive hormonal axis and the hormonal control on spermatogenesis, rather than by a direct toxic effect on the seminiferous tubules of the testes. In a previous study, Wong and collaborators (2004) discovered that cadmium, as well, caused changes on the blood-testis barrier before inducing vascular changes.

Effects of cadmium on the blood-testis barrier are well document in the literature (Hew et al., 1993; Chung and Cheng, 2001, Cheng and Mruk, 2002). In 1993, researchers proposed that cadmium may promote disruption of Sertoli cell

tight junctions (Hew et al., 1993). Chung and Cheng (2001) proposed that cadmium reduced tight junction proteins responsible for cell adhesion that participate in intercellular sealing (Cheng and Mruk, 2002) expression in Sertoli cells.

Cigarette Smoke

Reviewing another common environmental exposure, Vršanská and colleagues (2003) explained that in addition to being widely recognized as a health exposure hazard, smoking cigarettes also affected reproductive health. The link between smoking and female fertility disorders, including poor embryo development following in vitro fertilization treatment and even infertile offspring has been well established (Zenzes, 2000). Smoking has also been associated with delayed conception, in human females, (Baird and Wilcox, 1985) and a reduced number of retrieved oocytes leading to premature menopause (Bolumar et al., 1996).

Supporting the notion that smoking has contributed to the worldwide decline in semen quality; male studies that examined environmental factors and paternal fertility have demonstrated an association between cigarette smoke and sperm concentration. In 1992, Carlsen and collaborators systematically reviewed 61 studies of semen quality conducted over 50 years and found that mean sperm concentration worldwide fell by half from $113x10^6$/ml in 1940 to $66x10^6$/ml in 1990. In 1993, Giwercman et al. concluded that such a fast decline in semen quality was probably due to environmental, rather than genetic factors. The group suggested that in utero exposure to environmental oestrogens, pollution and

lifestyle exposures, including cigarette smoking were possible causes of this decline in quality (Giwercman et al., 1993).

In a 1996 paper, through meta-analysis of studies previously between 1981 and 1982, Vine showed that smokers' sperm concentration was on average about 15.0% lower than that of non-smokers. Two decades later, Lotti and colleagues (2015) confirmed that male smokers showed lower ejaculate and lower ultrasound-derived seminal vesicles volume, despite higher testosterone levels, when compared with non-current smokers.

Cadmium, a heavy metal previously discussed as an environmental exposure, is present in tobacco as well. Stassen et al. (1990) demonstrated that smoking cigarettes, and most likely second hand smoke inhalation, represented a primary source of inhaled cadmium. Investigators have hypothesized that second hand smoke has caused a significant decline in the fertility ability of men (Cheng and Mruk, 2012). Researchers explained that cadmium is a known teratogen and carcinogen that accumulates over a period of years and is easily incorporated in the reproductive tissues such as gonads and uterus (Pařízek et al., 1969; Hamada et al., 1998).

In a 2009 murine study, Oliveira and colleagues, reported that short term effects of cadmium resulted in an increased fraction of sperm with abnormal morphology, premature acrosome reaction, and reduced motility. Late term effects included a drastic reduction of sperm cell numbers and sperm motility, as well as, an increased detection of DNA fragmentation (Oliveira et al., 2009).

Previously, in a 2000 study, Telisman et al. reported decreased male fertility related with occupational exposure to cadmium. In addition, an association was observed, by Xu and colleagues (2003), between the presence of cadmium in seminal plasma and decreased sperm quality and increased sperm oxidative damage. In 2007, Ozmen and colleagues detected a cadmium based correlation between progressive motility and human sperm cells. In the same study, the group observed a relationship between DNA fragmentation and acrosome integrity in sperm cells exposed to cadmium (Ozmen et al., 2007).

The Centers for Disease Control and Prevention (2006) suggested that cigarette smoke leads to infertility through a combined effect of decreased sperm motility with active paternal smoking, decreased tubal patency with active maternal smoking, and/or second hand smoke exposure. The observed relationship between lifestyle exposures and the adverse effects on male reproductive health has increased the need for further smoking related studies.

Heat Exposure

Research has demonstrated that an increase in testicular temperature is considered another environmental exposure that has a negative effect on male fertility. In 2007, Shefi and investigators described that increased testicular heat, such as in saunas or hot tubs, elevated the testicular temperature and impaired sperm production. In 2014, Rato and colleagues further described such sensitivity to increased testicular heat, that even a sedentary lifestyle should be considered a potential confounder for reduced sperm count because of the increase scrotal heat.

Senger (1999) explained that researchers found that exposure of the scrotum to hot temperatures for periods of 16 hours a day did not influence the spermatozoal numbers. However, a reduction in motility and percentage of live spermatozoa occurred when the testes were heated for only eight hours per day. Additionally, the group observed that when 16 hours per day of heat was applied to the scrotum the survival of embryos produced by normal females was reduced (Senger, 1999).

Male Infertility Length with Current Partner

In a 2005 publication, Wright and colleagues were in agreement that the rising number of children born after assisted reproductive technology is a reflection of the increasing number of couples seeking treatment for infertility. Recent studies have emerged stating that underlying infertility and time to pregnancy is a proposed risk factor for adverse pregnancy outcomes, independent of maternal age (Zhu et al., 2006).

About 10.0 to 20.0% of couples who are trying to become pregnant experience a waiting period to pregnancy longer than 12 months, which is the clinical definition of infertility in most industrialized countries (Juul et al., 1999). A 2000 study suggested that the older a man was the higher his infertility length with current partner (ILCP) or the longer it may take his partner to conceive, regardless of her age (Ford et al., 2000). The authors' claimed that women with partners five or more years older have less chance of conceiving within a year of trying than those whose partners are the same age, or younger (Ford et al., 2000).

The investigation, through a large population study, was conducted by Ford and colleagues in 2000 to evaluate the effect of paternal age on time to conception. The group reported that older men were significantly less likely than younger men to impregnate their partners in ≤6 or in ≤12 months. Interestingly, the average male age in which fertility expressed a significant decline was similar to that of previous research studies on women.

The independent effects of female ageing on fertility among the general population have been clearly demonstrated using donor insemination as a model (Federation CECOS et al., 1982). After age 30 years, a slow decline has been observed in females and it rapidly increased after 40 years; now the main limiting factor in the treatment of infertility (Hull et al., 1996; Templeton et al., 1996; Spandorfer et al., 1998). However, a decline in male fertility with age has never been confirmed or quantified by studies in the general population. Male fertility remains difficult to measure directly except in small and atypical populations such as couples attending fertility clinics. In addition, quantification of the effect of advanced male age is confounded by many other factors. Weinstein and Stark (1994) acknowledged that studies on the ageing of a male can be compromised by the ageing of his partner and/or the decline in coital frequency associated with prolonged co-habitation.

Ford and colleagues (2000) took those effects into consideration and after adjustment the results of their study demonstrated a statistically significant increase with advancing male age in the proportion of couples who took longer than 6 or 12 months to conceive. The average age of the men who took >6

months to impregnate their wives was 31.8±5.75 years compared with 30.8±5.27 years in men who took ≤6 months (Ford et al., 2000). In addition, the group discovered that men who took >12 months were also significantly older, 32.6±5.91 years, than men who took ≤12 months, 30.9±5.32 years (Ford et al., 2000).

From their 2000 study, Ford et al. reported that the odds of conceiving within six months of trying decreased by 2.0% for every year that the man is older than 24 years, and for conception within a year decreased by 3.0% for each year. The group came to the conclusion that the probability of an ultimately fertile couple taking >12 months to conceive nearly doubles from approximately 8.0% when the man is <25 years to approximately 15.0% when he is >35 years. The authors proposed that these results suggested a larger decline in male fecundity with advancing age than reported in earlier population studies.

There are a number of researchers that believe time to conception can be a useful epidemiological marker of fertility. Even so, it has to be used with caution because it ignores couples who fail to conceive and is subject to a number of sources of bias (Baird et al., 1986; Joffe and Li, 1994; Olsen et al., 1998; Spira, 1998; Tuntiseranee et al., 1998).

Significance of Male Infertility Research

It is important to note that 10.0% of couples attempting to have children suffer from infertility. According to a 2001 report from Guzick and colleagues, each year 1.2 million men seek help for infertility and 15.0% are accurately diagnosed with male factor infertility using a semen analysis. Seventy percent of

IVF cycles fail for reasons unknown. However, sperm is suspected to contribute significantly to this failure rate.

Heightened by current societal trends to delay parenthood, understanding the effects of male age on semen quality is especially relevant for men attending reproductive clinics. The reliance on modern technologies, especially among marginally fertile older men is steadily increasing (Schmid et al., 2007). Although, ICSI and IVF have enhanced the probability of achieving fatherhood, they also circumvent the natural barriers against fertilization by damaged sperm (Maher et al., 2003; Singh et al., 2003). Further research needs to be done to better understand the mechanisms that are involved in the decline of sperm quality and fertilization capabilities, with regards to advanced male age and environmental lifestyle exposures.

Reproductive Clinician Perspective

Due to the increased proportion of infertile couples adopting to conceive by in vitro fertilization, predicting outcomes is of ever increasing importance in the human fertility industry (Brincat et al., 2014). Clinicians agree, since pregnancy rates following IVF are still quite low, prognostic information is very helpful in clinical decisions. Brincat and colleagues (2014) explained that although significant research is available on the maternal influence, updated male factor infertility research is still relatively unavailable for clinician application.

A new concern addressing the human fertility industry is the lack of adequate information clinicians are giving to patients on male infertility factors. Investigators from various industries have conveyed a number of sources that

have led to this phenomenon in male fertility treatment. However, most clinicians agree on two major issues that hinder sufficient male participation and treatment; a lack of consistent and current male infertility data and the deficiency in personal medical information provided by male patients. Both of these concerns are significant contributors to less efficient clinical treatment of male infertility factors. Additionally, this supports the current discrepancy in the treatment process experienced individually by the male and the female within the infertility couple.

Researchers have explained that male patients appeared to be more likely to confide in and desire information and emotional support from infertility clinicians rather than from friends or mental health professionals (Glover et al., 1994; Hammarberg et al., 2010; Brucker and McKenry, 2004). Therefore, if patients are not getting adequate information on male infertility factors from their doctor visits they are highly unlikely to learn about fertility issues and lifestyle exposure factors through additional resources.

In a 2010 review of research, Dancet and colleagues discovered that in only 5.0% of studies concerning patients' perspectives on fertility care focused specifically on the male perspective. Throsby and Gill previously broached this subject in a 2004 study of the male experience and ART. The pair reported that the normative assumption about the importance of child bearing and rearing coupled with the focus of ART treatment on the woman's body have reduced the visibility and awareness of the male experiences of childlessness (Throsby and Gill, 2004).

Coupled with the abundant research on the natural behavioral differences observed between males and females, male reproductive studies have reinforced that men are more likely to avoid issues concerning their personal infertility. Hjelmstedt et al. (1999) found that significantly more men, approximately 50.0%, than women had not shared their infertility issues with another person. The group interpreted the study results as a reflection of the inherent male frustrations of being in a situation that is poorly understood and in which assured treatments are availably researched or described to the patient.

Greil and colleagues (2010) explained that men can be affected by infertility in several ways: through receiving a diagnosis of their own infertility, through being the partner of a woman who is infertile, or through being part of a couple with unexplained infertility. Although the psychological and social aspects of infertility, fertility treatment with ART, and infertility-related childlessness have been investigated comprehensively in women, the psychosocial consequences of infertility for men are less well understood (Greil et al., 2010). Therefore, with continued research, clinicians would have the ability to provide male patients with answers to the unknown factors and encourage improvements in their environmental lifestyles to enhance their personal reproductive success.

CHAPTER III: RETROSPECTIVE STUDY EXAMING MALE INFERTILITY; THE ASSOCIATION BETWEEN AGE, ENVIRONMENT, AND REPRODUCTIVE SUCCESS IN MALE PATIENTS THAT HAVE PARTICIPATED IN ASSISTED REPRODUCTIVE TECHNOLOGY

Introduction

In high-income countries, approximately 15.0% of heterosexual couples experience difficulties conceiving when pregnancy is desired, and in up to half of these couples, infertility is attributable to the male partner (Skakkebaek et al., 1994). Inhorn (2009) explained that in the world's resource constrained low and lower-middle income countries, the prevalence of infertility in couples is thought to be higher because of undetected and untreated reproductive-tract infections.

Research has proposed several theories on the exact mechanisms that are responsible for the age-related decline in male fertility. Yet, scientists are still unable to determine exact mechanisms that are to be blamed (Belloc et al., 2014). One obstacle to overcome is the natural heterogeneous nature of human sperm. Semen samples in humans are so variable that it has been difficult for investigators to define the exact mechanisms.

In addition to age, there are a growing number of male infertility factors that are receiving new interest from reproductive scientists. Recent research has reported controversial results on a number of possible male infertility factors such as; lifestyle exposures, BMI, and ILCP. However, the fact that the exact associations have not been found has done nothing to deter the ever rising popularity of assisted reproductive physiology treatments.

In 2011, a total of 151,923 ART procedures performed in the U.S. were reported to the Centers for Disease Control and Prevention (2012). These procedures resulted in the birth of 61,610 infants. In line with the rising ART procedures is the rising age of couples receiving infertility treatment.

A number of studies demonstrated that as female age increased, fertility rates decreased. Yet, little research attention has been focused on male related infertility factors. The few studies performed; claim that 40.0 to 50.0% of infertility problems experienced by couples originate from paternal factors. Considering that statement, male age and lifestyle need to be equally factored into the equation.

To date, research has identified these potential predictors: fertilization, age, reactive oxygen species, sperm quality parameters, and DNA fragmentation (Brincat et al., 2014). Predictors under investigation which have shown promising signs in data include: folate and homocysteine, anti-mullerian hormone measurement, environmental factors, body mass indexes, smoking, male age, stress, some subsets of antisperm antibodies, and epigenetic features (Brincat et al., 2014). However, no definitive predictive value of these and more male infertility factors have been isolated to accurately gauge reproductive success.

In a 1998 review, Tarin and colleagues explained that late spermatids and immature and mature spermatozoa do not have a DNA repair system. Moreover, the activities of antioxidant enzymes within the seminal plasma and spermatozoa from older men may be reduced, thus, contributing to the reason that spermatozoa of older men are more vulnerable to mutational changes.

These detrimental effects can lower the overall sperm count by stopping or slowing the actual production of sperm. The fewer the normal sperm that are present the less likely it is that the oocyte will be successfully fertilized. In addition, these adverse factors can cause decreased mobility, abnormal morphology, and/or other DNA damage. Many defects can contribute to impaired fertilization so ideally, the fewer sperm with problems, the more likely that the sample has good fertilizing potential (Menkveld and Kruger, 1995).

Male and female gametes each contribute 23 DNA storing chromosomes at fertilization. Therefore, any damage, breaks, or changes in DNA can result in the inability of the sperm to fertilize the oocyte. If the altered sperm cell does in fact fertilize the oocyte, then development of the embryo and fetus may be affected, causing miscarriage or possible health problems for the offspring (National Institute for Occupational Safety and Health, 1996).

Investigators perform semen analysis testing to diagnose and manage male infertility. However, the limitations of conventional testing methods have been well documented. The most commonly evaluated parameters are sperm volume, sperm morphology and sperm motility. Recently, a number of more sophisticated assays including; measurements of sperm DNA fragmentation rates, seminal oxidative stress, and antioxidant capacity have been identified (Barazani et al., 2014). However, they are not a standard in the evaluation of male infertility and many clinics do not test for such additional parameters.

Previous research has led to the need of implying a female cutoff age. As researchers are discovering semen quality is a large contributor to reproductive

success, further research will help to determine if cutoff ages need to be applied for males as well. Theoretically, if cutoff age limits were currently mandated under federal law, having age limits for females and not males would be considered sex discrimination. We can only assume that these issues and more will arise in the next decade.

Purpose and Objectives of the Study

This study was designed to investigate the effect of male age and environmental lifestyle factors on the reproductive outcome of patients who had previously participated in clinical fertility treatments. Prior research has demonstrated that advanced female age, among other female factors, is directly related to reproductive success. However, there has been a limited amount of research performed on the effects of advanced paternal age and male lifestyle factors on reproductive success. This study was designed as part of a two component project to address these influences.

Reproductive clinicians are being confronted with elevated pressure to produce successful fertility treatments for an increasing number of couples. As older age and environmental factors are being shown to reduce reproductive success rates, more information regarding this problem is necessary to implement more efficient practices of infertility treatment programs.

In the first study, reproductive success will be determined through a combination of outcome variables; semen analysis, including sperm volume, concentration, morphology, motility, and percent normal, and biochemical

pregnancy rates. The type of reproductive treatment administered will be recorded as IVF or ICSI.

The original purpose of this study was to evaluate possible correlations between male age and environmental lifestyle factors that posed a threat to male fertility. Originally, data on approximately 50 variables were attempted for collection from male electronic medical records; they were subsequently narrowed down based on various factors. The selected variables were isolated for two specific reasons; they were listed in the review of literature and they provided the most consistent data available in the male medical charts. The following list of specific objectives was designed by the researcher to evaluate any possible correlations:

1. To describe clinical and study sample data on infertility patients who have participated in retrospective ART treatments from 2011 to 2014 at a private human fertility clinic in the southwestern region of the United States on the following selected characteristics.
2. To determine if there is a relationship between the age and reproductive success rate of male infertility patients who have participated in ART treatments at a private human fertility clinic in the southwestern region of the United States as measured by a randomized retrospective evaluation of patient charts from 2011 to 2014.
3. To determine if there is a relationship between environmental lifestyle factors such as; male occupation, cigarette smoking, alcohol use, caffeine use, recreational drug use, hot/bath tub use, steroid use, high fever,

and/or chemical exposure, and reproductive success rate of male infertility patients who have participated in ART treatments at a private fertility clinic in the southwestern region of the United States as measured by a randomized retrospective evaluation of patient charts from 2011 to 2014.

4. To determine if there is a relationship between male body mass index (BMI) and reproductive success of male infertility patients who have participated in ART treatments at a private fertility clinic in the southwestern region of the United States as measured by a randomized retrospective evaluation of patient charts from 2011 to 2014.

5. To determine if there is a relationship between infertility length with current partner and reproductive success in male infertility patients who have participated in ART treatments at a private fertility clinic in the southwestern region of the United States as measured by a randomized retrospective evaluation of patient charts from 2011 to 2014.

Materials and Methods

Research Design

The primary purpose of this retrospective study was to determine if a relationship existed between advanced male age infertility factors and human reproductive success. After a review of the literature, the researcher chose to investigate three additional variables of controversy; male environmental lifestyle exposures, male BMI, and infertility length with current partner.

A retrospective study was conducted using anonymous data from patients who had previously participated in ART cycles. The samples included patients

treated from 2011 to 2014 at a private human fertility clinic in the southwestern region of the United States. Reproductive success was determined by assessing biochemical pregnancy rates and semen analysis.

The study was designed to address research findings from the review of literature and available patient data observed retrospectively. The following objectives were written in the form of research hypotheses to be tested:

1. Human males who have previously participated in clinical ART treatments from 2011 to 2014 will have decreased reproductive success rates as male age increased in these patients.
2. Human males who have previously participated in clinical ART treatments from 2011 to 2014 will have decreased reproductive success rates based on cigarette smoking.
3. Human males who have previously participated in clinical ART treatments from 2011 to 2014 will have decreased reproductive success rates based on alcohol usage.
4. Human males who have previously participated in clinical ART treatments from 2011 to 2014 will have decreased reproductive success rates based on caffeine usage.
5. Human males who have previously participated in clinical ART treatments from 2011 to 2014 will have decreased reproductive success rates as BMI levels increased in these patients.

6. Human males who have previously participated in clinical ART treatments from 2011 to 2014 will have decreased reproductive success rates as ILCP increased in these patients.

The dependent variables for this study were biochemical pregnancy rates and semen quality. Commonly evaluated semen parameters used to determine quality were; volume, concentration, motility, progressive motility, percent normal, and total motile sperm per specimen. Independent variables were male age and male environmental lifestyle factors such as; urological history, chemical exposure, male BMI, and infertility length with current partner.

Approval to conduct this study was obtained from the Louisiana State University Institutional Review Board, IRB# E8892 (Appendix A). Exemption was granted under the compliance of the guidelines for human retrospective studies. In addition, a request to waive patient consent forms was approved by the IRB for reasons that; the type of research presented no risk of harm to the subjects and there would be no way to trace the study data back to the individual participant(s) (Appendix B). In addition, there was a high possibility that some participants may not be accessible to sign the consent waiver as they were no longer patients of the clinic.

In August of 2014, a study collaboration agreement was discussed amongst the primary researcher and the laboratory director of a private human fertility clinic located in a largely populated area of the southwestern region of the United States. The specific clinic was selected for a number of reasons; the utilization of advanced electronic patient records, proven clinical success rates, a

high volume of diversified patients among a large area, and the consistency of the same technician performing semen evaluations and assisted fertility procedures.

In a positive association, The Society for Assisted Reproductive Technologies (SART) has reported more than once that the current study clinic consistently outperformed the national average. According to the recently released SART report on 2013 IVF cycles, or procedures involving IVF, the study clinic once again achieved one of the highest IVF success rates in the nation. In 2013, SART reported that the national average pregnancy of women under the age of 35 was 47.7% (Society for Assisted Reproductive Technology, 2013); the collaborating clinic reported an average of 58.3% (Society for Assisted Reproductive Technology, 2013). The clinic also reported success rates for women ages 35 to 37 at 43.2% and women between the ages of 38 to 40 at 59.5%, significantly higher than respective national figures, 39.2% and 28.5% (Society for Assisted Reproductive Technology, 2013).

With IRB and private clinic approval, the retrospective study was designed around initial clinical patient consultations. Each patient and their partner were asked to complete an electronic questionnaire. Both males and females were requested to examine approximately 50 questions on lifestyle factors; such as physical characteristics, medical history, fertility history, urological factors, infertility length with current partner, gender specific questions, and a history of various exposures. The descriptive information collected from these

questionnaires was incorporated into each new patient's secured electronic medical records.

Population and Sample

The population of the study was defined as male infertility patients and their partners who have previously participated in fertility treatment(s). The patients specifically went through ART treatment cycles from 2011 to 2014, at a private human fertility clinic in the southwestern region of the United States. In a retrospective analysis of the total population, the average female age was 35 years old and a pregnancy rate of 55.0% was observed by the researcher.

A sample of 132 randomly selected female patients was obtained from the original population of ART participants. Matching male partner data was subsequently collected from patient electronic medical records. The random sample of females was each assigned an identification number. Then male partner information was collected and assigned a corresponding identification number.

Instrumentation and Data Collection

In September of 2014, e-mail correspondence between the Researcher, the Reproductive Laboratory Director, and the Medical Director was exchanged regarding the study proposal and IRB approval. On December 16, 2014 the researcher e-mailed the fertility clinic a signed copy of a confidentiality and nondisclosure agreement designed specifically for this study (Appendix C). In addition, a copy of the IRB approval form and project summary were forwarded to the clinic. On January 20, 2015, at the invitation of the lab director, the

researcher visited the human fertility clinic to further present the purpose and rationale of the retrospective study and to discuss lab protocol. In agreement, the group decided that the most useful and accurate patient information would be obtained from cycles performed from June 2011 to December 2014. This time period would provide the most complete electronic medical records. At the conclusion of this meeting, the confidentiality and nondisclosure agreement was verified and additionally signed by the Medical Director.

The initial study proposal was to include a multi-center population of male patients; however, the group decided that the diversity of the population in this particular clinic, the normal to above normal average success rates, and the consistency of using the same evaluator would produce generalizable results. Additionally, in the review of literature, the researcher examined a large population of studies that came from individual clinics.

Over the next two months, the researcher traveled to the fertility clinic for three to four days at a time to securely collect patient data. This was done to ensure that the sample patient identification was kept anonymous. The laboratory directory generated a discrete list of all female patients who had partaken in a treatment cycle or multiple cycles in the previous six years, listing only patient identification number, retrieval date, female age, peak E2 at hCG, βhCG levels on day 14, and the observation of a gestational sac(s).

Variables associated with male infertility factors were initially collected following a review of related literature and analysis of the specific clinic survey questionnaire. Original variables collected were; male and female age and

biochemical pregnancy status. From the female patient medical charts fertility partners were identified as male, female, or donor. The date and type of the ART treatment was collected in addition to the date of semen collection and characteristics analyzed. Descriptive male factors such as; height, weight, male BMI, infertility length with current partner, pregnancy history, medication history, and longtime illness history, were also collected. Urological and environmental lifestyle variables collected included; male occupation, patient and partner smoking history, male caffeine and alcohol consumption, history of vasectomy, hormone treatment, impotence, testicular abnormalities, white blood cell count in semen, male recent high fever, male hot/bath tub use, steroid use, recreational drug use, and male chemical exposure.

Through an extensive review of the female electronic patient records of the randomly selected sample, available data was collected and couples were recorded by their corresponding sample numbers. Patient numbers were recorded into an excel spread sheet with female age and the presence of a biochemical pregnancy. Male information was added to the document and identification numbers were recorded as one complete sample. For example, a female sample number 111 was correlated to male partner number 111.111 and their data were recorded jointly as a sample couple.

Data Collection

Pregnancy status based on gestational sac(s) presence, were observed by an ultrasound technician at an eight week gestational sonogram of the female patient. The presence of one or more gestational sacs confirmed a biochemical

pregnancy. For this study one or more gestational sacs were recorded as a positive biochemical pregnancy and zero sacs observed were recorded as non-pregnant.

Date of semen collection and cycle treatment was recorded as month, day, and year. This allowed the researcher to calculate the correct male age at the time of semen collection. Abstinence time period before collection was recorded in number of days. Semen parameters were recorded from male electronic patient records. Semen evaluation scores were previously recorded by the clinical andrologist, using the WHO reference values of human semen characteristics 5th edition in combination with the Kruger Strict Criteria for; volume, concentration, motility, progressive motility, Strict morphology percent normal, number of round cells, pH, and total motile sperm per specimen sample (Table 3.1).

Height and weight were recorded as self-reported by the patient. Male BMI levels were automatically calculated by the clinic evaluation form or by the researcher from supplemental male patient data. BMI levels were recorded as underweight, normal weight, overweight, or obese. According to the CDC (Centers for Disease Control and Prevention, 2015), overweight refers to an excess amount of body weight that may come from muscles, bone, fat, and water, whereas obesity refers to an excess amount of body fat.

Table 3.1. Semen was analyzed using the following parameters and range of references (Cooper et al., 2010).

Semen Parameters	Normal Range of Reference
Volume (ml)	1.5 to 5.0 ml
Concentration (million/ml)	>=15 million/ml
Motility (%)	>=40.0%
Progressive Motility	>=3 on 0 to 4 scale
Strict Morphology Percent Normal	>=4.0%
Round Cells	<1 million
Ph	7.2 to 8.5
Motile Sperm/Specimen	>=16 million

Length of infertility with their current partner was determined by the number of months without conception and/or live birth. When evaluating male BMI levels and ILCP, donor samples were automatically removed because no data was obtained for those patients. Additional samples were removed for ILCP in same sex partners.

Environmental lifestyle exposures were recorded to analyze data on occupation, male smokers, male alcohol use, male caffeine use, male recreational drug use, history of male hot/bath tub use, male steroid use, history of recent high fever, and male chemical exposure. In addition, male chart completion rate was recorded for the samples that were analyzed for the clinic evaluation. This data collection addressed two purposes of the study; one was to investigate the presence of a relationship between these variables and male

reproductive success and the second was to evaluate the amount of missing male data.

Occupation was originally recorded as specific job type and then grouped into categories of job exposure to possible harmful variables. This data was collected for two purposes as well. The first was an attempt to obtain a large enough sample size to evaluate a relationship between occupation and male reproductive success. The second was to again, identify the number of missing male sample data.

Cigarette smoking was recorded as smoker or non-smoker for the male patient and their partner. Alcoholic beverages were recorded as the number of drinks the male patient consumed daily, weekly, or socially. The number of caffeinated beverages was recorded as the amount consumed by the male patient per day, per week, or per month. Data was also recorded for male usage of recreational drugs, hot/bath tub use per week, recent high fever, steroids for body building, and chemical exposure.

History of sexually transmitted disease and treatment were recorded as the type of disease(s) and current status. Impotence, history of hormone treatment, history of vasectomy, and surgical history were recorded. In addition, undescended testicles, trauma to the testicles, painful swelling or torsion of the testicles were recoded. History of white blood cells in the semen and history of prostate infection were recorded for male patients with available data. Herbal remedies or vitamins, medications, and long standing medical illness(s), as well as, special diet were recorded for male patients when data was available.

The researcher then reviewed the expansive data set for inadequate study samples. Samples were removed if they fell into one or more of the following categories; canceled cycle, no partner identified, and/or the patient quit. Due to the nature of the fertility industry, incomplete patient data is commonly seen, especially in males. Based on the specific data available for study participants, the researcher elected to create two sub-samples containing separate variables. Although, a large number of participants were in both sub-samples, a few additional samples were included or excluded based on their available data. To increase clarity for the reader, the sub-samples will be identified as biochemical pregnancy sample and semen sample from this point forward.

The biochemical pregnancy sample consisted of 102 sample couples. Twenty samples were excluded from the original sample for the following reasons; the sample consisted of couples with missing biochemical pregnancy data, the use of biopsy ICSI for patient ART procedure, missing male age data, and some samples of donor sperm. In the cases of donor sperm, same sex female couples were removed; however, same sex male couples were retained if one of the partners semen sample was used for treatment. The average female age in the biochemical pregnancy sample was 35 years old, the average male age was 38 years old, and biochemical pregnancy was recorded at 44.0%. Males ranged in age from 26 years old to 52 years old and females ranged in age from 24 years old to 44 years old.

In the semen sample, 104 patients were included based on their available data of semen analysis parameters. Since the current study defined reproductive

success in two ways; biochemical pregnancy observation and semen analysis, 21 male patients who lacked pregnancy data but contained complete semen sample data, where included in this population. The sample variables included; semen characteristics, male age, lifestyle exposures, urological history, BMI, and ILCP. Average male age and range did not change among samples.

A normal characteristic of the human fertility industry is the lack of complete data for collection and/or analysis of patient samples. The semen sample contained more missing variable data than the biochemical pregnancy sample. However, that was to be expected due to the fact that not all sperm donors reported abstinence length and certain semen evaluation parameters are not available once the sperm has been frozen and thawed.

Data Analysis

The unit of observation, for evaluating male infertility factors, was reproductive success, defined by two dependent variables; biochemical pregnancy and semen analysis. As previously mentioned, patients were divided into two sub-samples, n = 102 and n = 104, based on the availability of patient data. Both samples were used to evaluate the relationships of each objectives listed below. Due to lack of response data, sample size ranged in some of the variables.

Since, the availability of male data unreported was a variable of interest; the researcher identified the samples that had missing data due to collection constraints, not because of the lack of patient response. Those cases were not included in specific variable evaluations. In contrast, data that were obviously

missing because of a lack of patient response were retained to evaluate the response rate of male data collection.

The researcher developed the following objectives to accomplish this portion of the study:

1. To describe clinical and study sample data on infertility patients who have participated in retrospective ART treatments from 2011 to 2014 at a private human fertility clinic in the southwestern region of the United States on the following selected characteristics:

- Male and Female Age
- Biochemical Pregnancy Rates
- ART Procedure Implemented
- Semen Analysis
- Male BMI Rates
- Male Occupation
- Patient and Partner Smoking History
- Male Alcohol Usage
- Male Caffeine Consumption
- Male Hot/Bath Tub Exposure
- Male Chemical Exposure
- Male Medication Usage
- Male Infertility Length with Current Partner

IBM SPSS was used to run descriptive statistics for Objective 1. Data was collected upon initial random sampling from the study clinic female patient list;

subsequently, male data was collected after being matched with the correct female identification number. Mean, sample size, standard deviation, frequency, and normal distribution were used to characterize the study samples.

Descriptive statistics were also utilized to identify data with relevant sample sizes for further analysis, additionally bringing to light the number of incomplete male patient records. The mean male age at collection was 38 years old and using IBM SPSS was found to be normally distributed (Appendix F.1).

Biochemical Pregnancy Sample

Biochemical pregnancy rate for the sample of 102 patients was 44.0%, regardless of female age or art procedure. A one-sample t-test (Appendix F.2) showed no significant difference between the average pregnancy rate of the study sample, 44.0%, and the average ART pregnancy rate of the national population, 39.0% (Society for Assisted Reproductive Technology, 2013). The average female age of the 102 study sample patients was 34.5 years old. Females ranged in age from 24 to 44 years old with a standard deviation of 4.6 years.

Of the 102 samples, 72 contained data for the type of ART treatment performed, IVF or ICSI. This difference in sample size is from research collection constraints not missing data. ART procedures were evaluated for frequency and were found randomly equivalent; 36 IVF cycles and 36 ICSI cycles. The mean for the 72 samples was .50 and the standard deviation was .50. For IVF, 15 patients were recorded as not pregnant and 21 patients were recorded as biochemically pregnant. For ICSI, 14 patients were recorded as not pregnant and 22 patients

were recorded as biochemically pregnant. The type of treatment cycle was held constant for Objective 2, after the researcher observed a common trend in positive pregnancy data in older males and ICSI rates.

Semen Sample

When descriptive statistics were obtained for the 104 samples of semen analysis data, the average male age at collection was 38 years, ranging from 26 to 52 years old with a standard deviation of 6.14 years. The semen descriptions that follow will identify the sample size for each variable, as well. Once semen is frozen and then thawed out, certain characteristics cannot be obtained or no longer provide relevant results. Different sample sizes were seen for abstinence, progressive motility, percent normal, and pH, due to donor records and frozen/ thawed semen records.

Abstinence contained 93 samples that reported an average time period of three days. Patients reported a range in abstinence from 1 to 21 days and a standard deviation of 2.58 days. Out of all of the 104 samples reported, average semen volume was 2.6 ml. Semen volume ranged from .2 to 8.5 ml with a standard deviation of 1.62 ml. Semen concentration was also available for 104 samples and demonstrated an average of 39.14 ml/million, with a notable standard deviation of 32.20 ml/million and a reported range of 0 to 144.00.ml/million. Average motility, also with104 samples recorded, was 47.1%. Range in motility varied from 0 to 84.0% with a standard deviation of 18.44. Out of 100 samples, progressive motility showed a 2.7 average measured on a scale

of 0 to 4. The progressive motility range reported included the entire scale from 0 to 4 with a standard deviation of .85.

For percent normal, 90 samples were available with a reported average of 5.8%, a reported range of 0 to 15%, and a calculated standard deviation of 3.64. With 90 samples, pH average was recorded at 7.6. The minimum pH level recorded was 7.2 and the maximum pH level recorded was 7.8 with a standard deviation of .16. Out of 104 samples, total sperm per specimen showed an average of 52.8 million, with a range of 0 to 403.2 million, and a standard deviation of 61.8. Out of the 72 samples that contained biochemical pregnancy rates in combination with semen factors, a 60.0% average was recorded, which is abnormally high compared to the national average but not compared to the clinic average.

Male BMI data was available for 70 respondents with the average level being 29, an identical reflection of the national average of male BMI rates which are currently reported at 29 (Flegal et al., 2012; Ogden et al., 2012), confirmed by a one-sample t-test (Appendix F.3). The range in BMI level recorded was 19.93 to 45.23 with a standard deviation of 5.9. The researcher converted BMI to categorical data for better analysis of the results. BMI levels were distributed throughout four commonly observed groups as seen in Table 3.2. The four levels of 70 samples were coded and first evaluated for frequency and then for bivariate correlating relationships using SPSS.

Sixty participants contained data recorded on ILCP, averaging 38 months. With a reported maximum ILCP at 210 months and a minimum at 0 months the

standard deviation was calculated at 39.8 months. When the researcher evaluated the large range in data, outliers and unqualified data samples were removed. Still, the remaining data left to be evaluated contained 49 samples with an average ILCP of 42 months, a range of 12 months to 150 months and a standard deviation of 32.7. The researcher chose to categorize the data into groups to try and obtain a better correlation analysis. In addition, let it be noted that a high lack of male patient response to this variable led to approximately 50.0% of the data being obtained from female partner electronic medical records.

Table 3.2. Male BMI Level Classification (Centers for Disease Control and Prevention, 2015).

BMI Classification	
Below 18.5	Underweight
18.5 to 24.9	Normal Weight
25.0 to 29.9	Overweight
30.0 or Greater	Obesity

Occupation was first evaluated for frequency; and, of the 37 samples recorded out of a sample size of 83, only three occupations listed more than one frequency. Seven of the male participants filled out evaluations but were identified for specifically skipping that question. The reason the researcher recorded it as a skipped question instead of missing data was to further identify the relationship between male response rates and possibly sensitive but informative questions.

For that reason, occupation data was combined into five very subjective categories of possible work hazards or work exposure. To be clear, this was just an estimated distribution into categories created by the researcher's review of related literature and the researcher's evaluation of the occupation job description. There were no distinguishing differences among the samples when occupations were placed into the categories; inside versus outside work, positive or negative chemical exposure, sedentary or active occupation, and high stress as opposed to low stress occupations. Therefore, occupation was not further analyzed.

With a sample size of 83, frequency of partner smoking did not contain enough variance to analyze. Only two cases of partner smoking were observed and 23 samples were missing. The frequency of male patients who smoked was 51 non-smokers, four smokers, 5 to 10 cigarettes per day, and 28 missing samples.

Out of 83 samples observed for male alcohol use, 48 samples responded. The highest frequency was from 18 patients who recorded 0 drinks per day. The second highest frequency, with 10 samples, was one drink socially. Responses ranged from 0 drinks to 14 drinks socially. The researcher removed extreme outliers and coded alcohol usage as yes or no. Alcohol use was defined as 1 to 5 drinks socially. Caffeine use contained 49 responses and 29 of the samples recorded daily use. These variables were correlated to determine if a relationship existed between male consumption and reproductive success.

With 50 valid responses out of 83 samples, there were only seven reports of bath/hot tub use. Frequency of chemical exposure had little information. Three samples out of 83 reported chemical exposure to toluene, refrigerant, and pesticides/herbicides. There was not enough information to be analyzed for either variable.

2. To determine if there is a statistically significant relationship between male age and reproductive success rate of male infertility patients who have participated in ART treatments at a private human fertility clinic in the southwestern region of the United States as measured by a randomized retrospective evaluation of patient charts from 2011 to 2014.

The researcher used a point-biserial correlation coefficient to evaluate biochemical pregnancy rate, a dichotomous variable, in relation to age and semen characteristics. In addition, the type of ART treatment was analyzed for correlation among pregnancy rate and male age using a point-biserial correlation coefficient. Pearson's product moment correlation coefficient was used to analyze possible relationships between semen characteristics and male age.

3. To determine if there is a statistically significant relationship between environmental lifestyle factors such as; male occupation, smoking, alcohol use, caffeine use, recreational drug use, hot/bath tub use, steroid use, high fever, and/or chemical exposure and reproductive success rate in male infertility patients who have participated in ART treatments at a private fertility clinic in the southwestern region of the United States as

measured by a randomized retrospective evaluation of patient charts from 2011 to 2014.

A frequency analysis was performed using data from the semen sample group. This section contained the majority of the variables from the patient questionnaire and the response rates were not consistent. If there was not a substantial amount of relevant data, the variable was not further analyzed.

Initial analysis of male patient data revealed a 62.3% response rate of the male patients' history of smoking. Five percent of male patients reported smoking 5 to 10 cigarettes per day and 61.4% reported not smoking.

Alcohol usage and amount displayed and 69.0% response rate. Caffeine usage and amount showed a 64.0% response rate. The response rate for recreational drug use was 64.0%, 1.0% of the sample reported cannabis use and all other respondents reported no drug usage.

With a 63.0% response rate, medication initially looked to have valid data. From the response group, the percent of samples that reported no medication usage was 65.0%. Samples in the response group reported usage of 4.0% for each of the following; Adderall, antidepressant, hormone related medication, and asthma medication. The use of blood pressure medication was reported by 14.0% of the response sample. In addition, the use of herbs or vitamins was reported by 31.0% of the response group.

Urological variables presented similar response rates, and due to lack of variance in the data, a number of variables were not further analyzed. White blood cells in the semen had a response rate of 64.0%, of which 98.0% reported

having no history and 2.0% reported yes to having had white bloods cells in semen. The response rate for prostate infection data was 65.0%, no history of infection was reported by 96.0% and 4.0% reported having had a prostate infection. In the data on recent high fever, 49.0% samples reported no and 51.0% had no response. STD data displayed a 58.0% response rate, of that 81.0% responded with no history, 14.0% reported having been treated for an STD, and 5.0% skipped the question. Difficulty with erection had a response rate of 62.0%; of the samples, 90.0% said no, 8.0% said yes, and 1.0% skipped the question. Difficulty with ejaculation had a response rate of 62.0%; of the samples, 70.0% said no, 8.0% responded yes, and 22.0% skipped the question.

The remaining urological variables did not have enough variance in the data to further explore; hormone treatment, vasectomy, surgery to the testicles, undescended testicles, trauma to the testicles, and painful swelling of the testicles. Overall these variables had an average of 60.0% for their response rates. However, the majority of the responses stated no issue, and the average answer of yes was approximately 2.0%.

The following variables were removed based on low frequency rate of response and/or invalid data. Male diet was removed because out of a 50.0% response rate, one sample recorded a special gluten free diet and the remaining samples reported no special diet. Steroid usage was removed because the entire 52.0% response rate samples reported no use. With a 56.0% response rate for hot/bath tub use, 78.0% responded no usage.

4. To determine if there is a statistically significant relationship between BMI and reproductive success rate in male infertility patients who have participated in ART treatments at a private fertility clinic in the southwestern region of the United States as measured by a randomized retrospective evaluation of patient charts from 2011 to 2014.

When categorical BMI data was analyzed the sample of 70 consisted of; 0% underweight males, 13.5% normal weight males, 34.6% overweight males, and 19.2% of males were classified as obese. BMI classification levels of male patients were correlated with biochemical pregnancy, male age, ILCP, and semen samples to meet this objective.

5. To determine if there is a statistically significant relationship between infertility length with current partner and reproductive success rate in male infertility patients who have participated in ART treatments at a private fertility clinic in the southwestern region of the United States as measured by a randomized retrospective evaluation of patient charts from 2011 to 2014.

Upon initial analysis, the researcher used a normal distribution analysis and observed a maximum outlier of 210 months and a minimum outlier of 8 months, both were removed from the sample group. Additionally, as human fertility treatment procedures increase in popularity, we must consider that not all patients participate in treatment cycles due to infertility issues. Therefore, the samples that recorded less than 12 month and were seeking treatment for things such as gender selection of the offspring were removed as well. Two samples

were removed with an answer of zero, stating that the couple was preparing for male infertility, and one sample with an un-reversed vasectomy was removed.

A Pearson's correlation coefficient in SPSS was used to evaluate for a relationship between ILCP and male reproductive success. A point-biseral correlation coefficient was used to analyze for a biochemical pregnancy relationship and ILCP. No significant correlation was observed among any of the variables. Further analysis could either stop here or ILCP could be converted to categorical data. The researcher decided to further analyze ILCP by forming three categories; ILCP 12 to 24 months, ILCP 25 to 48 months, and ILCP ≥49 months.

Statistical Analysis and Findings

In this section, results of correlational analyses are reported for the dependent and independent variables. The research hypotheses are listed at the beginning of each respective subsection, and are followed by an explanation of the statistical analyses. The final section will contain an overview of the results in a discussion.

1. Human males who have previously participated in clinical ART treatments from 2011 to 2014 will have decreased reproductive success as male age increases in these patients.

The researcher ran a point-biseral correlation coefficient to evaluate if a significant relationship existed between biochemical pregnancy status and male age at collection. At a value of r = -.196, a statistically significantly negative

correlation was exhibited between biochemical pregnancy and advanced male age at a confidence level of .05 (Table 3.3).

Table 3.3. Pearson product point-biseral correlation coefficient table exhibits a statistically significant negative relationship between biochemical pregnancy and age of male infertility patients who have participated in ART treatments at a private human fertility clinic from 2011 to 2014 (Appendix F.4).

Point-Biseral Correlation Coefficient

		Male Age at Collection	Biochemical Pregnancy
Male Age at Collection	Pearson Correlation	1	-.196*
	Sig. (2-tailed)		.049
	N	102	102
Biochemical Pregnancy	Pearson Correlation	-.196*	1
	Sig. (2-tailed)	.049	
	N	102	102

* Correlation is significant at the 0.05 level (2-tailed).

As the researcher expected to find, female age demonstrated a negative correlation with biochemical pregnancy rate and a positive correlation with male age at time of collection. When comparing female age and biochemical pregnancy rate the researcher performed a point-biseral correlation coefficient generating a Pearson's r value of r = -.209 at a confidence interval of .05. The relationship between female age and male age at the time of collection was analyzed using a Pearson's correlation coefficient. With a value of r = .549 at a confidence interval of .01, the two variables exhibit an obvious significant relationship (Table 3.4).

Table 3.4. Pearson product correlation coefficient exhibits a statistically significant relationship between biochemical pregnancy rate, female age, and male age of infertility patients who have participated in ART treatments at a private human fertility clinic from 2011 to 2014.

The Pearson Product Moment Correlation Coefficient

		Female Age	Biochemical Pregnancy	Male Age at Collection
Female Age	Pearson Correlation	1	-.209*	.549**
	Sig. (2-tailed)		.035	.000
	N	102	102	102
Biochemical Pregnancy	Pearson Correlation	-.209*	1	-.196*
	Sig. (2-tailed)	.035		.049
	N	102	102	102
Male Age at Collection	Pearson Correlation	.549**	-.196*	1
	Sig. (2-tailed)	.000	.049	
	N	102	102	102

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

Figure 3.1 demonstrates the decreasing percentage rates of biochemical pregnancy success as male patient age increases. Results identified a 62.0% biochemical pregnancy rate for male patients' age 26 to 30 years old. Male patients' age 31 to 35 years old revealed a 59.0% rate, 36 to 40 years old a 32.0% rate, 41 to 45 years old a 36.0%, and 46 to 56 years old a 30.0% rate.

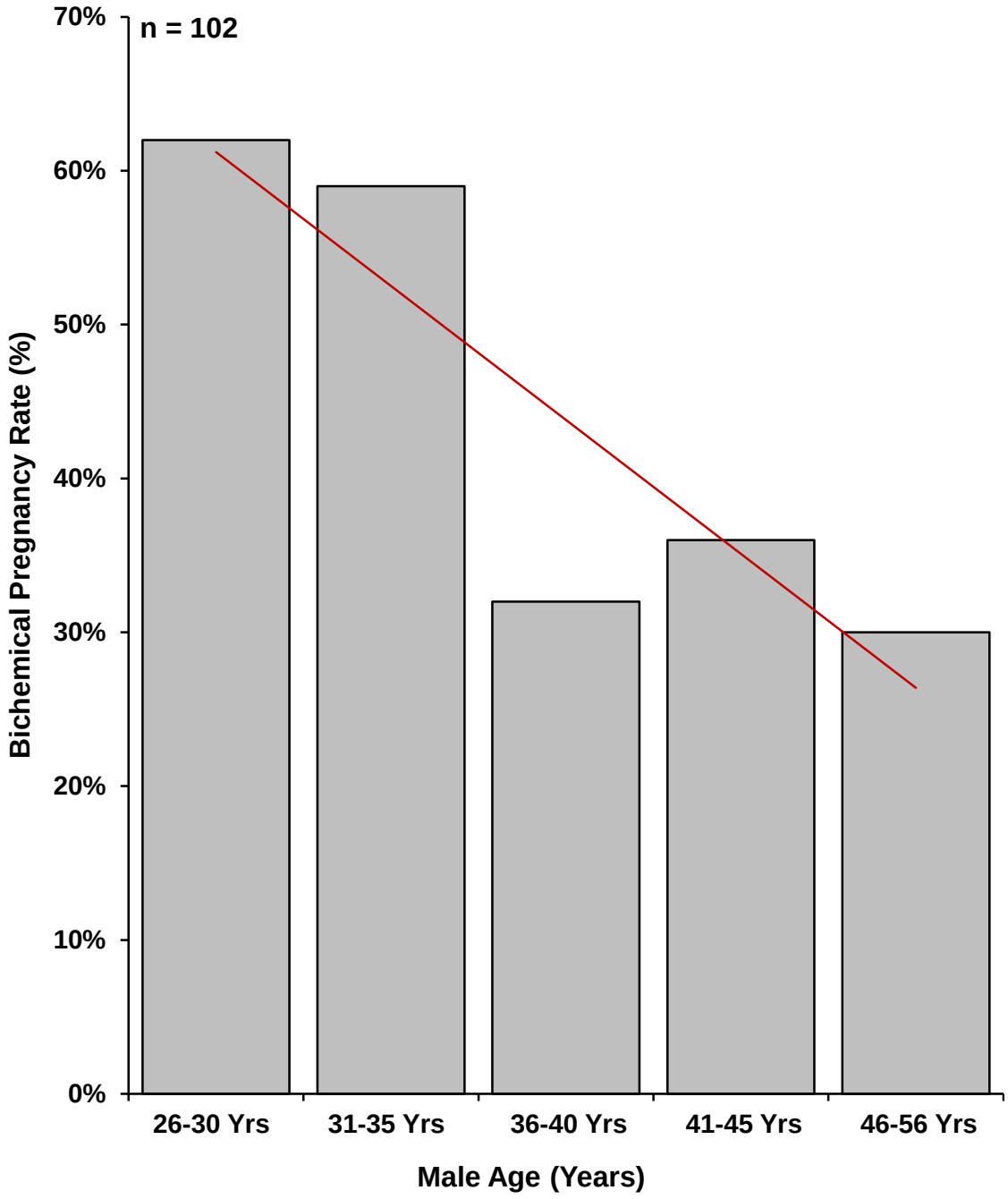

Figure 3.1. Biochemical pregnancy percentage rates of male infertility patients who have participated in ART treatments at a private human fertility clinic 2011 to 2014.

Previous observations led the researcher to further evaluate the relationship between male age and the type of treatment used for assisted reproduction. Table 3.5 displays the frequency distribution of ART procedures performed and recorded from the retrospective study sample. The researcher ran bivariate correlations for each of the two ART treatments in relation to biochemical pregnancy. Holding the ART treatment group constant for IVF (n = 36), a point-biserial correlation was performed to determine if a significant relationship between IVF biochemical pregnancy rates and advanced male age existed. Reporting a value of r = -.491, IVF biochemical pregnancy rates and advanced male age demonstrated a statistically significant negative correlation at a highly significance level of .01 (Table 3.6).

Table 3.5. Frequency distribution of retrospective study sample ART procedures performed at a private infertility clinic from 2011 to 2014.

ART Procedure	Frequency	Percent	Valid Percent	Cumulative Percent
IVF	36	35.3	50.0	50.0
ICSI	36	35.3	50.0	100.0
Total	72	70.6	100.0	

Table 3.6. Pearson's point-biseral correlation coefficient table exhibits a statistically significant negative relationship between IVF biochemical pregnancy and age of male infertility patients who have participated in ART treatments at a private human fertility clinic 2011 to 2014 (Appendix F.5).

Point-Biseral Correlation Coefficient

		Biochemical Pregnancy	Male Age at Collection
IVF Biochemical Pregnancy	Pearson Correlation	1	-.491**
	Sig. (2-tailed)		.002
	N	36	36
Male Age at Collection	Pearson Correlation	-.491**	1
	Sig. (2-tailed)	.002	
	N	36	36

** Correlation is significant at the 0.01 level (2-tailed).

The researcher went on to run an additional point-biserial correlation to investigate if a significant correlation existed between ICSI biochemical pregnancy rates and advanced male age. This time holding ART treatment constant for ICSI, the results recognized an insignificant p value of r = .153.

Table 3.7 demonstrates the results of descriptive statistics used to analyze semen characteristics from male patients included in the retrospective study sample. Using a point-biserial correlation coefficient to evaluate for relationships among biochemical pregnancy and each semen parameter, no significant correlations were found. However, a Pearson's coefficient demonstrated a statistically significant negative correlation between male age and volume,

r = -.338 at a confidence level of .01. Pearson's correlation coefficient also exhibited a statically significantly negative correlation among male age and progressive motility at r = -.202 with a confidence level of .05. Table 3.8 displays a complete list of the significant relationships found among male patient semen characteristics.

Table 3.7. Descriptive statistics of semen characteristics collected retrospectively from study sample male patients that participated in private clinical infertility treatment cycles from 2011 to 2014.

Characteristics	Mean	Std. Deviation	N
Male Age (years)	37.7	6.1	104
Abstinence (days)	3.0	2.6	93
Volume (ml)	2.7	1.6	104
Concentration (million/ml)	39.1	32.2	104
Motility (%)	47.0	18.4	104
Progressive Motility	2.7	0.9	100
Percent Normal (%)	5.8	3.6	89
Total Motile Sperm (specimen/million)	52.8	61.8	104

Table 3.8. Statistically significant bivariate correlations among semen parameters collected retrospectively from study sample male patients that participated in private clinical infertility treatment cycles from 2011 to 2014.

Correlations	Pearson's Correlation *r*	Significant (2-tailed)	N
Male Age/ Volume	-.338**	.001	104
Volume/Total Motile Sperm	.399**	.001	104
Male Age/Progressive Motility	-.202*	.043	100
Progressive Motility/Motility	.769**	.001	89
Progressive Motility/Concentration	.500**	.001	100
Progressive Motility/Percent Normal	.288**	.007	89
Progressive Motility/Total Motile Sperm	.379**	.001	100
Progressive Motility/Abstinence	-.219*	.035	93
Motility/Abstinence	-.340**	.001	93
Motility/Concentration	.436**	.001	104
Motility/Percent Normal	.416**	.001	89
Motility/Total Motile Sperm	.476**	.001	104
Concentration/Total Motile Sperm	.704**	.001	104
Concentration/Percent Normal	.269*	.011	89
Percent Normal/Total Motile Sperm	.321**	.002	89

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

2. Human males who have previously participated in clinical ART treatments from 2011 to 2014 will have decreased reproductive success rates based on cigarette smoking.

Using a point-biserial correlation coefficient a statistically significant positive relationship was observed between male smokers and sperm concentration at a p value of r = .313 with a confidence level of .05. In addition, using the same correlation coefficient, a statistically significant correlation between smoking and progressive motility was exhibited at r = .294 with a confidence level of .05 (Table 3.9). Using a cross tabulation table (Table 3.10) with Cramer's V coefficient exposed a statistically significant correlation between male smokers and difficulty with ejaculation at a value of r = .465 with a confidence level of .01 (Table 3.11).

3. Human males who have previously participated in clinical ART treatments from 2011 to 2014 will have decreased reproductive success rates based on alcohol usage.

Social alcohol usage was defined once the results were analyzed. Male patient social alcohol usage was considered an average consumption of 1 to 5 drinks socially (Appendix F.6). No significant correlation was found in male patients between consumption of alcohol socially and biochemical pregnancy. However, when evaluating for male age and semen characteristics related to social drinking, several significant correlations were identified. Using a Pearson's coefficient, male social alcohol usage and semen volume displayed a negative statistically significant correlation at r = -.304, with a confidence interval of .05.

Table 3.9. Pearson's point-biseral correlation coefficient table exhibits a statistically significant relationship between smoker, semen concentration and semen progressive motility of male infertility patients who have participated in ART treatments at a private human fertility clinic 2011to 2014 (Appendix F.7a and F.7b).

Point-Biseral Correlation Coefficient

		Smoker	Concentration	Progressive Motility
Smoker	Pearson Correlation	1	.313*	.292*
	Sig. (2-tailed)		.020	.036
	N	55	55	52
Concentration	Pearson Correlation	.313*	1	.545**
	Sig. (2-tailed)	.020		.000
	N	55	83	79
Progressive Motility	Pearson Correlation	.292*	.545**	1
	Sig. (2-tailed)	.036	.000	
	N	52	79	79

* Correlation is significant at the .05 level (2-tailed).

** Correlation is significant at the .01 level (2-tailed).

Table 3.10. Frequency distribution of retrospective study sample male patient smokers and difficulty with ejaculation presented in a Cramer's V correlation coefficient contingency table.

Difficulty with Ejaculation*Smoker Contingency Table

		Smoker		
		Non Smoker	Smoker	Total
Difficulty with Ejaculation	No	34	2	36
	Yes	2	2	4
	Skipped	11	0	11
Total		47	4	51

Table 3.11. Cramer's V correlation coefficient exhibits a statistically significant relationship between male patient smokers and difficulty with ejaculation.

Cramer's V Correlation Coefficient		Value	Approx. Sig.
Nominal by Nominal	Phi	.465	.004**
	Cramer's V	.465	.004**
N of Valid Cases		51	

** Correlation is significant at the .01 level.

In addition, when using Pearson's r correlation to compare male social alcohol usage and total motile sperm per specimen a statistically significant negative relationship was observed at a value of r = -.293, with a confidence interval of .05 (Table 3.12).

4. Human males who have previously participated in clinical ART treatments from 2011 to 2014 will have decreased reproductive success rates based on caffeine usage.

No significant correlation was found among the relationship of caffeine and biochemical pregnancy. However, a single statistically significant relationship was observed correlating BMI and caffeine using a Pearson correlation, with a value of r = .367 at a confidence level of .01.

Table 3.12. Pearson product correlation coefficient exhibits a statistically significant relationship between biochemical pregnancy rate, female age, and male age of infertility patients who have participated in ART treatments at a private human fertility clinic from 2011 to 2014.

The Pearson Product Moment Correlation Coefficient

		Social Alcohol Usage	Volume	Total Motile Sperm/Specimen
Social Alcohol Usage	Pearson Correlation	1	-.304*	-.293*
	Sig. (2-tailed)		.035	.043
	N	48	48	48
Volume	Pearson Correlation	-.304*	1	.342**
	Sig. (2-tailed)	.035		.002
	N	48	83	83
Total Motile Sperm/ Specimen	Pearson Correlation	-.293*	.342**	1
	Sig. (2-tailed)	.043	.002	
	N	48	83	83

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

5. Human males who have previously participated in clinical ART treatments from 2011 to 2014 will have decreased reproductive success rates as male BMI levels increased in these patients.

Using a point-biserial correlation coefficient there was no significant correlation observed between the BMI and biochemical pregnancy rate at a p value of r = -.018. A Pearson's correlation coefficient revealed a non-significant relationship between BMI and male age (r = .040), semen volume (r = .051), semen concentration (r = -.004), sperm motility (r = -.088), progressive motility (r = .042), percent normal (r = -.134), and pH (r = .008).

6. Human males who have previously participated in clinical ART treatments from 2011 to 2014 will have decreased reproductive success rate as infertility length with current partner increased in these patients.

Running a point-biseral correlation coefficient the researcher found no significant correlation between biochemical pregnancy rate and ILCP (r = .038). Via a Pearson's r correlation coefficient the researcher also discovered a non-significant relationship between ILCP and male age (r = -.193), semen volume (r = -.049), semen concentration (r = -.048), sperm motility (r = .058), progressive motility (r = .101), pH (r = .213), and total motile sperm per specimen (r = .021). However, a statistically significant correlation was observed between ILCP and percent normal semen at a value of r = .304, with a confidence level of .05, using a Pearson's correlation coefficient (Table 3.13).

Table 3.13. Pearson product correlation coefficient exhibits a statistically significant relationship between ILCP and percent normal semen of male infertility patients who have participated in ART treatments at a private human fertility clinic from 2011 to 2014.

The Pearson Product Moment Correlation Coefficient

		% Normal Semen	ILCP
% Normal Semen	Pearson Correlation	1	.304*
	Sig. (2-tailed)		.030
	N	70	51
ILCP	Pearson Correlation	.304*	1
	Sig. (2-tailed)	.030	
	N	51	60

* Correlation is significant at the 0.05 level (2-tailed).

Discussion

Assisted reproductive techniques have become increasingly popular with the current aging first time parental population. Although, one critical aspect that has been overlooked for years is the effect of male age on reproductive success. Previous decades of research have focused almost entirely on female infertility factors. However, recent studies have started to demonstrate that males may be affected in a similar manner. In a 2014 study, Brincat and colleagues claimed that abstract paternal influences on reproduction are significant in causing about half of infertile couples to turn to ART procedures.

Data from the current study demonstrated that male age does in fact have a significantly inverse relationship with biochemical pregnancy rate. The results demonstrated that as male age increased, fertility capabilities were shown to decrease. The older the male patient, regardless of their female partner's age,

the less likely they were to get a positive biochemical pregnancy result. This is not only important knowledge for clinicians; it is also important information to share with the population of couples who plan on starting families later in life.

Previous research has shown that advanced female age is correlated with reduced reproductive success. Therefore, the researcher expected a significant relationship to be demonstrated in older female patients and biochemical pregnancy rates. However, a noteworthy finding was the similarity of variance in the significant relationships discovered between biochemical pregnancy rate for female age and biochemical pregnancy rate for male age. In other words, the current results revealed a very similar relationship in individual contribution of advanced male age and advanced female age to reproductive success. Furthermore, when the relationship between female age and male age was correlated a highly significant relationship was found and should be further analyzed in future studies.

Females have continually been reminded of the biological time clock winding down on their reproductive years. However, current research has not only started to focus on age related factors of male infertility, but on the entire male lifestyle. Studies are beginning to acknowledge that age may not be the only newly recognized factor contributing to male infertility. Although researchers have accepted that there are more factors playing a role, determining those mechanisms has been elusive. This issue was reestablished by the results of the current study.

Therefore, the current study first chose to further examine the question of change in male reproductive capabilities as they increase in age. The controversy lies in determining if the factor contributing to decreased male reproductive success is in fact age. On the other hand, it is hypothesized that as males age they actually become more susceptible to environmental lifestyle factors that are hazardous to reproductive success.

Additionally, results from the present study demonstrated that in advanced age males, IVF pregnancy success rates significantly decrease. In today's fertility industry, ICSI has become a common procedure used by clinicians to treat older males or patients with known male infertility factors. The ICSI data from the retrospective study supported the use of that practice. When researching male fertility, consistency is extremely important considering there are so many variables when working with human semen. Therefore, the researcher felt it was beneficial to analyze the biochemical pregnancy rate while holding the ART treatment constant due to the increased efficiency of ICSI.

When evaluating semen results the researcher found a similar decrease in reproductive success as male patient age increased. Even with the small sample size of the current study, data confirmed that as male patient age increased semen volume decreased. The data also revealed an inverse relationship with advanced male age and progressive semen motility.

Once a human female reaches a certain age there is an abrupt decrease in reproductive capability. Conversely, newly focused research on the reproductive capabilities of the human male proposes a slow senescence of

reproductive decline as they age. The decrease in seminal volume observed in the current study further supports the indication of a significant decline in advanced age reproductive males.

Due to convenience and efficiency the fertility industry has adopted a simple one or two based semen analysis procedure. This leaves clinicians with an approximate 50.0% successful evaluation. The current study demonstrated an absolute need for more male data, more male awareness, and more male evaluation techniques. Changes will not happen overnight but the more frequently male fertility factors are researched, the more exposure they will receive.

When considering environmental threats, previous studies on the effects of smoking have reported reduced sperm concentration and motility in male cigarette smokers (Kunzle et al., 2003; Vine, 1996; Vine et al., 1996). The current study results reported a similar observation. Results demonstrated a decrease in semen concentration and progressive motility in those male patients who reported smoking 5 to 10 cigarettes a day. The results additionally concluded that male patients who reported smoking were also associated with ejaculation difficulty.

We must take in to consideration that since this was a retrospective study, missing data created a smaller sample size. When researching human semen characteristics it is more desirable to have a larger sample size for a more normalized average. In the current study, the sample of patients who demonstrated adequate data to compare for a relationship between smoking and

semen concentrations consisted of 55 males. For the relationship between smoking and progressive motility, 52 male patients reported adequate data. A future study with a larger sample size would be beneficial in examining the increasing age of male smokers in contrast to decreasing semen concentration and decreasing progressive motility.

The results of the current study also indicated that alcohol use in male patients was, in fact, correlated to impaired semen quality. Since, the impact of alcohol consumption on male fertility potential remains a controversial topic; the results of the current study are an important addition to the research. The results demonstrated that as male alcohol use increased seminal volume and total motile sperm per specimen showed a significant decline. Again the researcher observed two common semen variables that are reportedly affected by advanced male age as well. Further research is needed to test for repeatable results and correlations. Studies should be designed to evaluate the question of reduced semen quality from the effects of advanced male age or the susceptibility to alcohol consumption at advanced male age.

In the current research study, male BMI levels did not show a significant relationship with biochemical pregnancy rate and semen evaluations. In a previous study, Anifandis and colleagues (2013) reported similar results, finding no evidence of male BMI correlating with sperm parameters. However, in the Anifandis study, BMI did influence the quality of embryos produced in such a way that impacted pregnancy rate (Anifandis, 2013). The design of the current study would have failed to pick up on impaired embryo quality because retrospective

data was only collected on embryos that were fertilized and then transferred. Further studies need to be performed to isolate the effect of BMI on embryo fertilization and quality prior to transfer.

This raises the question, if high BMI levels are claimed to influence the quality of the embryo, how can researchers assume that BMI is not in some part responsible for alterations in semen quality. One consideration, of this assumption is that whatever factors are playing this detrimental role in embryo production, are not being tested for in a common semen analysis.

Another consideration is the difference between the effects of being overweight versus being obese. In 1998, the National Institutes of Health defined overweight as an excess amount of body weight that may come from muscle, bone, fat, and water. Obesity was defined as an excess amount of body fat. This suggests there may be a difference in semen quality expressed between the two BMI classifications. Research has demonstrated that increased lipid amounts have been shown to act on the male reproductive axis. It is possible that the key factor is the actual amount of fat.

In 2014, Rato and colleagues published a study that explained lifestyle and unhealthy eating can negatively affect spermatogenesis, both at central and gonadal levels. The group described that the overconsumption of high-energy diets (HED) altered the function of the male reproductive axis and consequently affects the testicular physiology, disrupting its metabolism and bioenergetic capacity. The group emphasized that disruption of the tightly regulated metabolic pathways leads to adverse reproductive outcomes, such as inefficient energy

supply to germ cells, sperm defects, or spermatogenesis arrest (Rato et al., 2014).

This leads to an interesting observation in the current study results. As previously mentioned, the researcher observed an unusual amount of male patients that very likely intentionally skipped the questions on difficulty with erection and difficulty with ejaculation. Further evaluation of these skipped samples, in addition to the samples who reported an erectile problem, revealed an average male patient BMI of 31.7, which is considered obese. If, in fact, this sensitive question is being skipped due to male discomfiture, we assume that there is more than likely some level of an erectile dysfunction problem with the male patient. Several investigators have reported that high BMI levels may reduce male fertility and have associated it with reduced semen quality and hormone alterations (Jensen et al., 2004; Kort et al., 2006; Fejes et al., 2005; Fejes et al., 2006). In addition, a 2004 study published by Fung and colleagues stated that overweight men may be at greater risk of erectile dysfunction which could lead to reduced fertility.

The final results of the current study found no significant relationship between ILCP and biochemical pregnancy rates or male patient age. The one significant correlation observed among semen characteristics was the relationship between ILCP and the percent normal semen. In 2006, Zhu and colleagues reported that underlying infertility and time to pregnancy is a proposed risk factor for adverse pregnancy outcomes, independent of maternal age. However, Weinstein and Stark (1994) acknowledged that studies on the

ageing of a male can be compromised by the ageing of his partner. Additionally, the group contributed a decline in coital frequency to be associated with prolonged co-habitation, (Weinstein and Stark) which could lead to a negative effect on the percent of normal semen.

There are a number of researchers that believe time to conception can be a useful epidemiological marker of fertility. Even so, it has to be used with caution because it ignores couples who fail to conceive and is subject to a number of sources of bias (Baird et al., 1986; Joffe and Li, 1994; Olsen et al., 1998; Spira, 1998; Tuntiseranee et al., 1998). Unlike the small sample size of the current study, Ford and colleagues (2000) found significant correlation among older men and ILCP by performing a large population study.

Unfortunately, by the time a couple gets to the fertility clinic today, most are at the point of wanting a child immediately, not wanting to change a lifestyle. As more research is done on male infertility factors, the issue is further uncovered. Therefore, this will only increase public exposure to the discussion on male infertility factors. Couples should be aware that the male partner is now realized to be a large contributor to reproductive success. Additionally, couples need to be informed that there are ways to proactively improve their own fertility chances, as well as, specific lifestyle changes that will accomplish improved reproductive success.

CHAPTER IV: CLINICIAN RESEARCH SURVEY METHOD: EXAMINING MALE INFERTILITY; THE ASSOCIATION BETWEEN AGE, ENVIRONMENT, AND REPRODUCTIVE SUCCESS IN MALE PATIENTS THAT HAVE PARTICIPATED IN ASSISTED REPRODUCTIVE THECHNOLOGY

Introduction

Male infertility is a term that was rarely discussed just a decade ago. Although, it is heard more frequently today, the term still carries considerable taboo behind its meaning. Decades of research have highlighted female as the focus of human infertility. It has been the female, not the male, who has been consistently studied on the successes and failures of reproduction. Early studies identified a number of female lifestyle factors that affected reproductive success. Subsequent research led to an establishment of assisted reproductive treatment methods, for specific infertility issues.

One form of assisted treatment, in vitro fertilization, is a common medical procedure practiced today. Although, not so long ago, it was a mysterious procedure that produced what were then only known as ‘test-tube babies.’ The same unknown label has been associated with male infertility today. What we currently view as foreign concepts may evolve into common practices, just as IVF demonstrated in a few short decades.

Reproductive research has progressed exponentially in the past 50 years and even still, we are continuously discovering additional factors. As couples wait longer to start families, an increased demand for research in the area of male

reproductive fertility is revealed. As our societal trends continue to evolve, advanced male age must be considered in the human infertility discussion.

Previous data has provided limited research on male infertility factors for a number of reasons. It is a continuous challenge to find adequate sample populations for human male studies in places other than infertility treatment centers.

Purpose and Objectives of the Study

The researcher selected an alternative approach by investigating the relationship of male age and environmental lifestyle factors on assisted reproductive technique success rates as observed from an infertility clinician's standpoint. The first objective was to compare the retrospective response rates of male patients in the previous study to clinicians' response ranks of the importance of specific male factors.

Next, was to compare the percent of retrospective male patient data available to male patient data observations made by clinicians in their own professional experience. The purpose of addressing missing information was to expose the amount and the importance of unreported male patient data. Through the retrospective study, the researcher wanted to take an inventory on the completion level of male patient records. Through the survey study, we wanted to gain data on the clinicians' experiences with incomplete male records. From the results, we expected to increase some understanding of the reasons behind missing data. Newfound information would help to encourage improved collection methods. In addition, the study was designed to help determine if there was a

pattern in the missing data, as well as possible ways to identify the purpose. For example, a male patient may not answer a sensitive question because he is uncomfortable with the topic or because he thinks the question is irrelevant to his fertility issues.

The goal of the survey study was not only to evaluate the actual opinion of professional clinicians, but to also compare the differences in opinions among clinicians. This will help to exemplify the variation level of existing standards among the infertility industry. Professional experience, from someone currently working in the industry, should provide a different perspective than the retrospective data results. The researcher designed the following objectives to describe study sample characteristics and to identify correlation data:

1. To describe the level of importance of male infertility variables that clinicians report as the most commonly observed in their professional experiences in comparison to the retrospective male response data collected on those same male infertility variables.
2. To describe the percentage of completed male medical records available from the retrospective analysis of data and the percentage of completed male medical records reported as seen by clinicians.
3. To determine if there is a statistically significant relationship among the opinions of clinicians who participated in the voluntary male infertility survey on topics such as; data availability and gender based ethical treatment of patients.

Materials and Methods

Research Design

The primary purpose of this study was to gain further insight on controversial male infertility factors from the unique perspective of reproductive clinicians. There is so much variability in practices and procedures throughout the infertility industry that a general consensus of which is the most effective remains unknown. The study was designed to collect clinical data in an unconventional method through the analysis of infertility professionals' responses. After a review of the literature, the researcher developed a series of questions based on current disputed male infertility factors. Gender related ethical practices present in today's industry were also addressed in an anonymous survey mailed to reproductive professionals.

The goal of the survey was to gauge male infertility factors from a different perspective. Collecting observations from existing professionals in the fertility industry, directed the survey identification of male infertility factors from a first-hand perspective. Survey results served to enhance research in this area by acknowledging the personal experiences of professionals. Combining multiple methods of evaluation; such as the clinician survey study in this chapter and the retrospective study in the previous chapter, we believe the results will help to create a better foundation for the basis of future research studies.

The unique approach of surveying scientists and physicians, through social science research techniques, while subsequently comparing their opinions to scientific data helped to create a more comprehensive method for data

collection. The first two objectives of this study were specifically identified by the researcher to describe the nature of the relationship between clinician opinions and the statistical data collected from the review of literature and retrospective study. This section of the research was designed to gain a broad sense of where the issues of male infertility stand currently.

While, the deficient amount of research in this area provides a limitless requirement for cause and effect studies to be performed. The approach of this portion of the study was to gain knowledge on the important male variables that are currently being observed in the industry. The next step on the continuum of research can then be based off of the results obtained from the clinician survey.

By addressing clinicians directly, the study had two goals in mind. First, to identify the variables of importance that practicing clinicians have reported from their treatment experiences of male infertility patients. The second was to gather information on the practices in male infertility treatment currently observed in the industry today. As the topic has continued to spread, a number of unknowns have been brought into the conversation. Not only is it important to identify the detrimental cause and affect variables on male reproductive success. It is also important to appreciate that the industry will be forced to re-evaluate ethical differences in treatment among male and female patients.

The researcher designed the following objectives and research hypothesis to describe study sample characteristics and to identify correlation data:

1. To describe the level of importance of male infertility variables that clinicians report as the most commonly observed in their professional experiences in comparison to the retrospective male response data collected on those same male infertility variables.
2. To describe the percentage of completed male medical records available from the retrospective analysis of data and the percentage of completed male medical records reported as seen by clinicians.
3. Clinicians who participated in the voluntary male infertility survey will demonstrate a negative correlation among their opinions of infertility topics such as; data availability and gender based ethical treatment of patients.

Approval to conduct this study was obtained from the Louisiana State University Institutional Review Board, IRB# E8894 (Appendix D). Exemption was granted under the compliance of the following guidelines; that participants cannot be identified, directly or statistically, and the responses/observations could not harm participants if made public. A waiver of signed consent was granted, with the inclusion of the survey instructions stating that participation is voluntary. The participants were informed that by completing the survey they were providing and documenting their consent.

Population and Sample

The population of the study was defined as clinical professionals who are currently associated with the human infertility industry. One outlet for survey

distribution, EmbryoMail, is a national human infertility membership group. Correspondence is directed through the group moderator and then forwarded on to members of the group. Membership is strictly for professionals within the infertility industry. Using this network, the researcher invited all qualified members to complete the IRB approved male infertility clinician survey (Appendix E).

In addition, approximately 20 Louisiana State University Alumni, currently working in the human reproductive industry, were used as another sample source. Members were emailed the same anonymous survey participation invitation. Results came from random voluntary participation. The researcher had no way of identifying participant personal information that was not asked by specific survey questions.

The study sample consisted of 53 voluntarily and anonymous survey participants. Clinicians who participated in the survey included 50.9% male professionals and 49.1% female professionals within the industry. Of the professionals that made up the study sample; 5.7% were Reproductive Endocrinologists, MD; 24.5% were Reproductive Physiologists, PhD; 9.4% were Andrologists; 56.6% were Embryologists; 1.9% were Urologists, MD; and 1.9% were Reproductive Technicians (Figure 4.1).

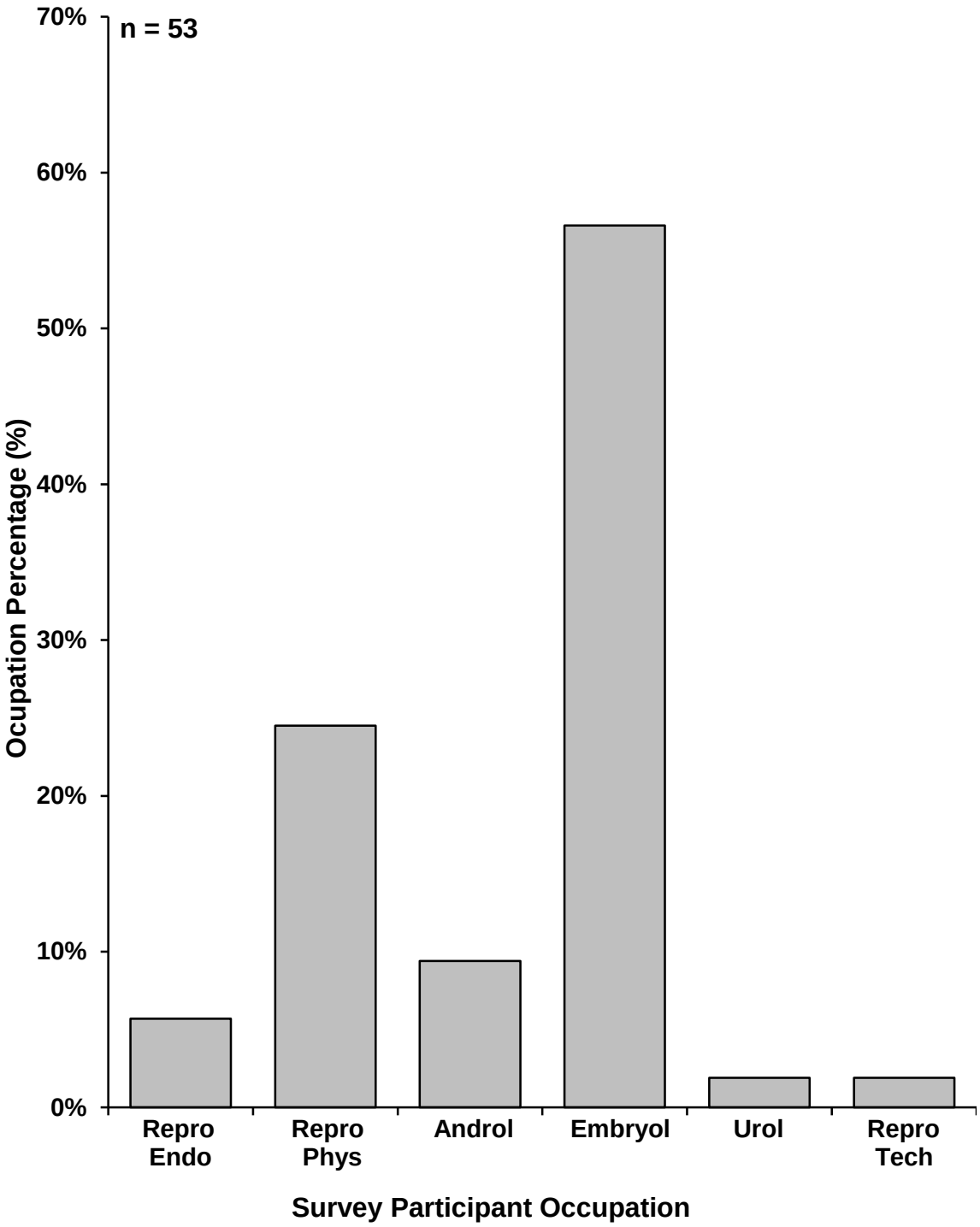

Figure 4.1. Percentage of occupations held by reproductive professionals who participated in the male infertility clinician survey.

Instrumentation and Research Procedure

The researcher used the program SurveyMonkey® to design and publish a 36 multiple choice questionnaire. Male infertility questions were based on topics from a review of the current literature. A pilot survey test was sent out to various colleagues in the area whose answers were not to be included in the results. On January 26, 2015, after positive confirmation of the instrument, an email invitation was sent to the EmbryoMail moderator and the group of LSU Reproductive Alumni.

Survey instructions were stated as follows: You are invited to participate in the 20 to 25 minute brief online survey. If you only have experience with some of the questions feel free to skip the ones that do not pertain to you and/or record an alternative answer. Any and all input is welcome in order to gain as much data from the human clinician side of the industry. Feel free to forward the survey link to other colleagues you think may be interested in participating. Thank you in advance for your assistance. To complete the survey click on the link below or copy and paste into browser: Please complete by February 16, 2015. https://www.surveymonkey.com/s/clinicalmaleinfertility.

As of February 16, 2015, survey data had been collected from 46 respondents. In a successful attempt to increase sample size, the researcher sent out a reminder e-mail extending the deadline to March 13, 2015. An additional seven participants were included. The final survey sample size totaled at 53 respondents.

Open ended answers and the suggestion for additional comments were offered in 14 of the survey questions. These questions were incorporated to gain additional feedback from the professionals on clinical practices that may not have been included in the survey answer options.

Survey responses and e-mails received by the investigator were opened with a secured internet connection. Participant information was received under an identification number with no way for the researcher to identify a participants' name or location. Additionally, a secure login was created by the researcher to upload, edit, and obtain results of the survey.

Data Collection

Participant responses were collected and identified by the order in which the survey was submitted. For example, the only information for Respondent #1 was that the participant began the survey 7:25 p.m. on January 26, 2015 and completed the survey at 7:40 p.m.; a total time of 15:19 minutes.

The researcher was able evaluate response data for each participant individually or as a whole sample group through the survey site. Both methods provided useful in monitoring progress and allowed for the researcher to identify additional comments from specific participants on their individual page(s). Survey results were recorded by gender, occupation, and specific question response, accessed only by the researcher.

The survey publisher program provided graphs based on descriptive data. However, to meet the objectives of the study, the researcher reviewed each survey sample and recorded their answers linking their profession and gender.

This allowed the researcher to focus on specific relationships that otherwise could not have been evaluated based on the design of the survey.

The researcher wanted address specific comparisons focused on occupation in relation to data accessibility and gender in relation to ethical questions. These survey questions can be viewed below in Table 4.1. In addition, the researcher wanted to evaluate the clinicians' opinions on, specific male infertility factors, patient information gathered by the specific clinic, and male infertility evaluation tests. The results of these questions were compared to corresponding data from the retrospective.

After realizing the amount of male data missing in the retrospective study patients, the researcher wanted to compare the clinicians' personal experience with this same issue. Percentages of completed male medical records were compared for both study samples. Comparing the results for association or disagreement allowed the researcher to evaluate the importance of missing male data from two separate perspectives.

Male medical chart completion percentage was evaluated for the observations given by the clinicians participating in the survey in percentage completions. In addition, the researcher evaluated the retrospective study medical charts for the amount of data missing. If an evaluation was completely missing that sample was considered 100% incomplete. If the evaluation was incomplete and/or missing data for three to five variables it was considered 25.0 to 50.0% complete. Complete evaluations and evaluations missing only one data point were considered greater than 75.0% complete.

Table 4.1. Male infertility clinician survey questions evaluated in relation to occupation and gender.

Survey Question (#)	Male Infertility Clinician Survey Questions
Question 1	What role do you play in reproductive health?
Question 2	Gender: Male or Female?
Question 4	In your current position, do you have access to and review all descriptive data for each male patient per cycle?
Question 7	In your current position, do you have access to and review all semen data for each male patient per cycle?
Question 16	In your current position, do you have access to and review all urological data for each male patient per cycle?
Question 19	In your current position, do you have access to and review all exposure data in each male patient per cycle?
Question 25	In your current position, do you have access to and review all medical history data in each male patient per cycle?
Question 32	Does reproductive healthcare need a better communication system allowing the physician and the laboratory physiologist to have equal access to the all of the male patient's exposure/environmental and past medical information per cycle?
Question 34	In your professional opinion do you find it unethical to provide fertility treatment for males after a certain age?
Question 35	In your professional opinion, do you find that we are adequately providing significant clinical information to older male patients on the risks and ethical issues of advanced age fertility treatments?

Statistical Analysis

The main unit of observation for this study was reproductive clinicians who voluntarily participated in an online survey. In some instances, these observations were also compared to results from the previous retrospective study. With Microsoft Excel 2010, descriptive statistics were used to determine the mean percentages of the most commonly seen male infertility variables observed by the professionals who participated in the survey. The percentage means were then used to rank the top five variables that were reported by clinician observations as important factors in male infertility.

Frequency rates and descriptive statistics were also used, in IBM SPSS Statistics 22, to identify percentage means of male data response rates from the retrospective study. The variables of importance identified by the clinician observations were then compared to the mean percentages of the same variables from the retrospective study. Since the study was measuring different rates/ranks, there were no statistical procedures performed other than descriptive statistics. Still, the researcher wanted to visualize the difference between what variables are of importance to clinicians and what variables are actually being reported by male patients.

In addition, descriptive statistics were used to determine the mean percentages of male record completion that were reported by survey participants. To compare the rates being observed by professionals in the industry and actual study data collected on completed male records, retrospective completion data was evaluated in association. Descriptive statistics had been previously applied

in the retrospective study to determine the frequency and percentages of completed male records reported. This allowed for a side by side comparison of industry observations and retrospective clinic data to be loosely and cautiously evaluated for similarities.

The researcher used Cramer's V correlation coefficient, in SPSS, to determine if there was a significant relationship between occupation and gender. In addition, Cramer's V coefficient was used to evaluate for a correlation between data access and clinician occupation. Data accessibility was examined for five different areas; descriptive data, semen evaluation data, urological data, exposure data, and medical data.

Again in IBM SPSS, the researcher used Cramer's V correlation coefficient to examine the relationship between gender and ethical male treatment practices such as; the need of a better clinician communication system and adequate information provided on advanced age male reproductive risks. When evaluating occupation and the need for a better clinical communication system, the researcher also used Cramer's V to determine a correlation. A Phi correlation coefficient was used to determine if there was a significant relationship between clinician gender and treatment cutoff age for males.

Results

This section begins with the results of survey questions that were not addressed in the statistical analysis. No statistical implications were made for these additional comparisons. However, the researcher thought it would be beneficial to the exploratory nature of the research design to evaluate clinicians'

opinions on these particular questions. In addition, response percentages from the survey study are compared, side by side, to analogous results from the retrospective study. The results of correlational analyses are then reported, implicating the type of relationship between surveyed clinicians' opinions, gender, and occupation for the dependent and independent variables.

Figure 4.2 demonstrates the opinion percentage means of surveyed clinicians on the effectiveness of basic semen analysis as a predictor of male infertility. Out of 53 participants, 67.9% responded that basic semen analysis was an effective predictor most of the time. Clinicians who reported semen analysis as only occasionally effective totaled 26.4% of the response group. Notably, only a small percentage of survey participants, 5.7%, reported that this analysis was effective all of the time.

Participating clinicians' opinions on the importance of DNA fragmentation as a predictor of male infertility are exhibited in Figure 4.3. The mean percentages of 52 respondents describe the professional opinions of those surveyed on this male infertility factor. Result demonstrated that 32.7% have no idea if DNA fragmentation was a predictor, 26.9% reported that occasionally it was a predictor, 23.1% rarely thought DNA fragmentation was a predictor of male infertility, 11.5% thought that most of the time this variable was an indicator, 3.8% responded never, and 1.9% DNA fragmentation always indicated male infertility.

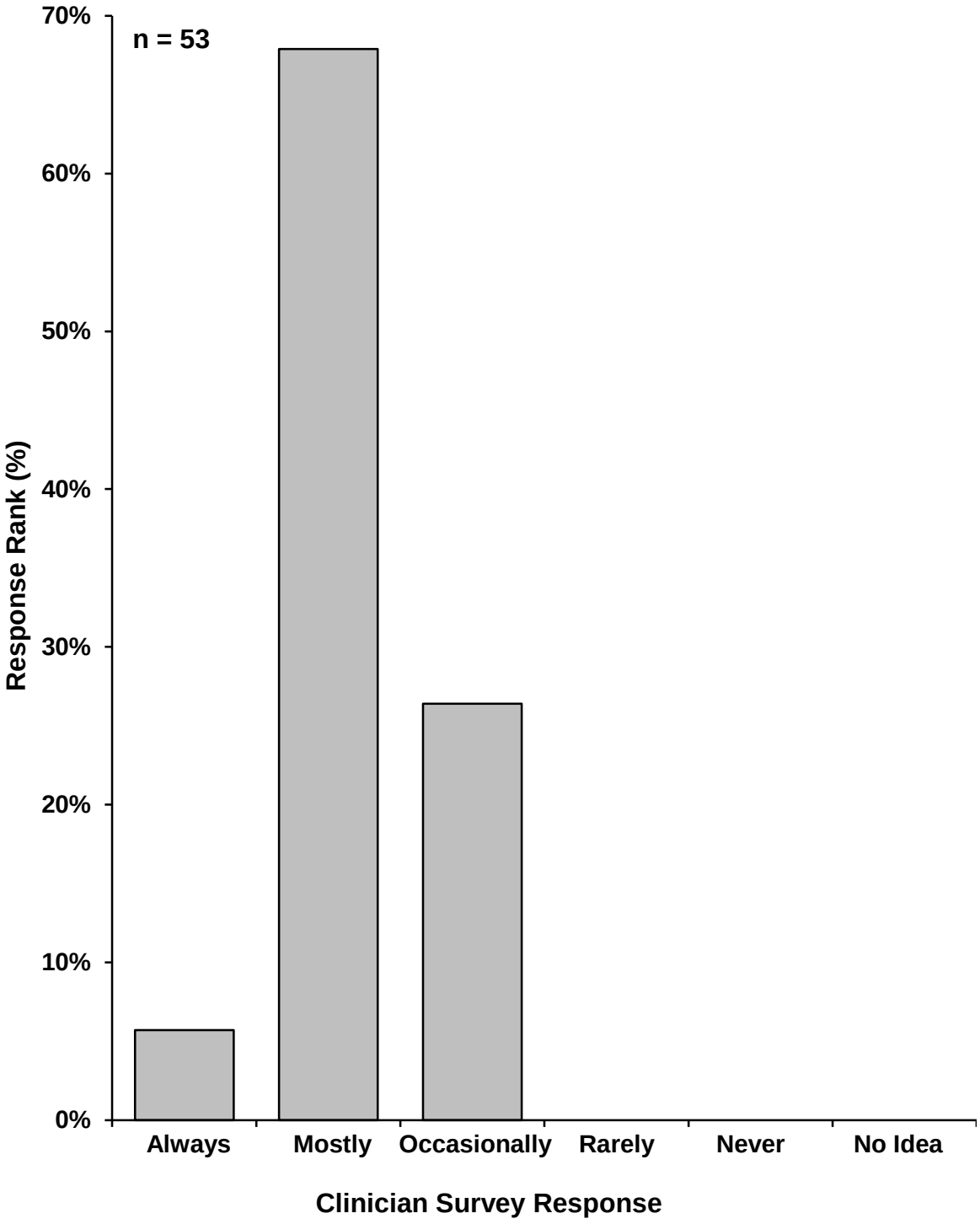

Figure 4.2. Percentage means of clinician responses on the importance of basic semen analysis as a male infertility predictor demonstrated by the response ranks for survey question number nine.

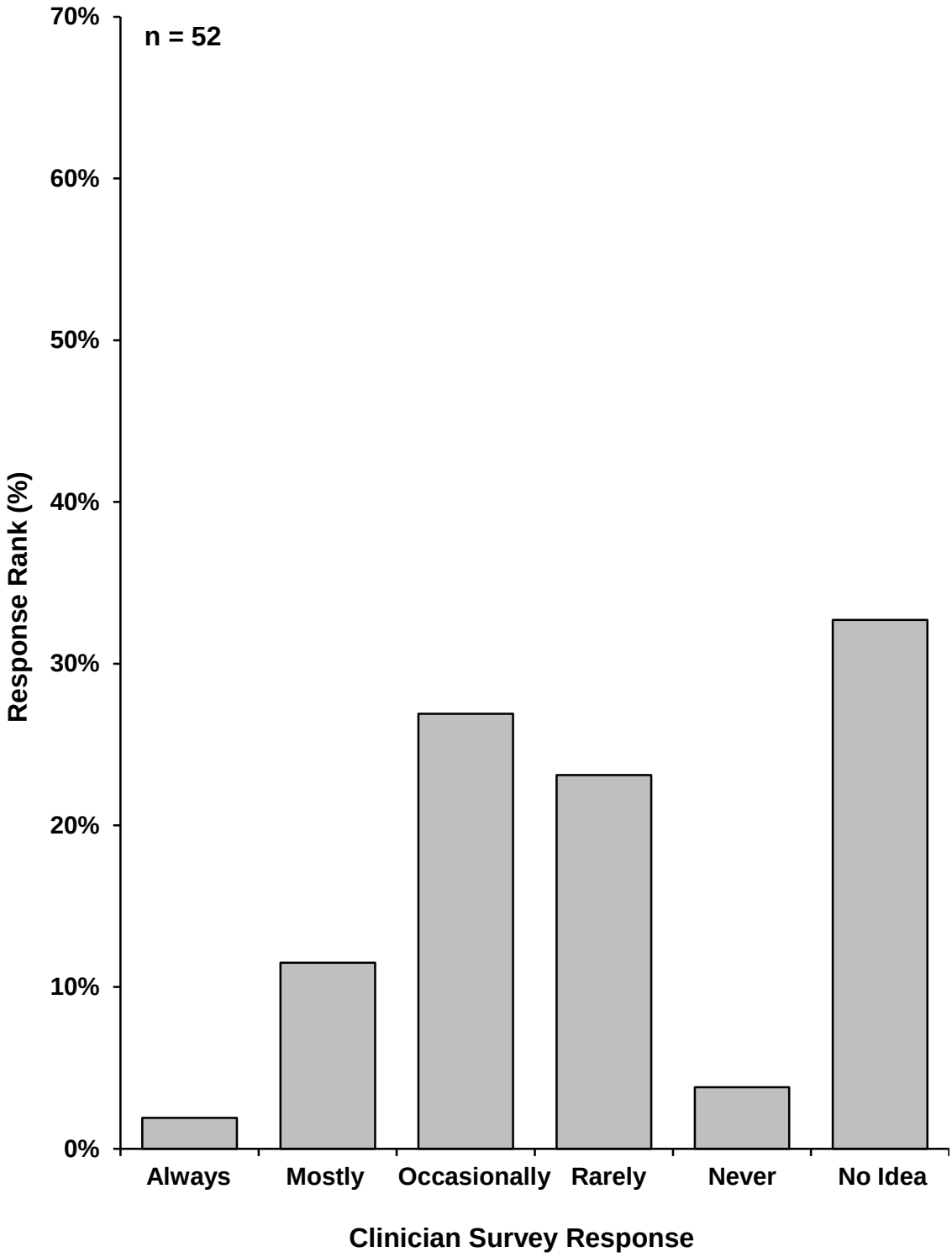

Figure 4.3. Percentage means of clinician responses on the importance of DNA fragmentation as a male infertility predictor demonstrated by the response ranks for survey question number ten.

However, when asked what semen parameters the clinic tested for, DNA fragmentation exemplified one of the lowest statics. The response rate for this survey question was 100%. As shown in Table 4.2, clinicians reported semen parameters tested at their clinics in the following order; volume, sperm count, sperm motility, and progressive motility were tested by 100% of the clinics. Progressive motility was reported to be tested by 90.6% of the clinics, while white blood cell count was tested 88.7% of the clinics. Less than half of the clinics, 30.2%, tested for DNA fragmentation and 13.2% tested for acrosome integrity.

Table 4.2. Percentages of semen parameters routinely evaluated as reported by the professional experience of surveyed clinicians.

Answer Options	Response Percent	Response Count
Volume	100.0%	53
Sperm Count	100.0%	53
Sperm Motility	100.0%	53
Progressive Motility	90.6%	48
Sperm Morphology	100.0%	53
White Blood Cell Count	88.7%	47
Acrosome Integrity	13.2%	7
DNA Fragmentation	30.2%	16
N of Valid Cases		53

The results of participant opinions on the significance of male age on impaired semen and/or sperm cells are presented in Figure 4.4. Approximately six percent (5.7%) of clinicians admitted to having no idea on the effects of male age as it related to impaired semen samples and no respondent (0%) believed male age was completely responsible. However, 58.5% were in agreement that male age had a somewhat significant effect on the integrity of semen and/or sperm cells. The remaining participant responses were closely divided, with 18.9% of clinicians believing that male age had a lot to do with impaired semen samples. On the other hand, 17.0% of clinicians believed that male age was a factor of little significance.

When clinicians were surveyed on the significance of genetic and epigenetic changes in sperm DNA, the results demonstrated an increased agreement in contribution on male infertility (Figure 4.5). Approximately 80.1% of professionals responded that genetics and/or epigenetics displayed somewhat or a lot of significance on male infertility, 41.5% and 39.6%, respectively. Additional results demonstrated that 9.4% of clinicians assumed that this variable had a little significance on male infertility, 7.5% believed that these changes were completely responsible for male infertility, and 1.9% had no idea of the significance as related to male infertility.

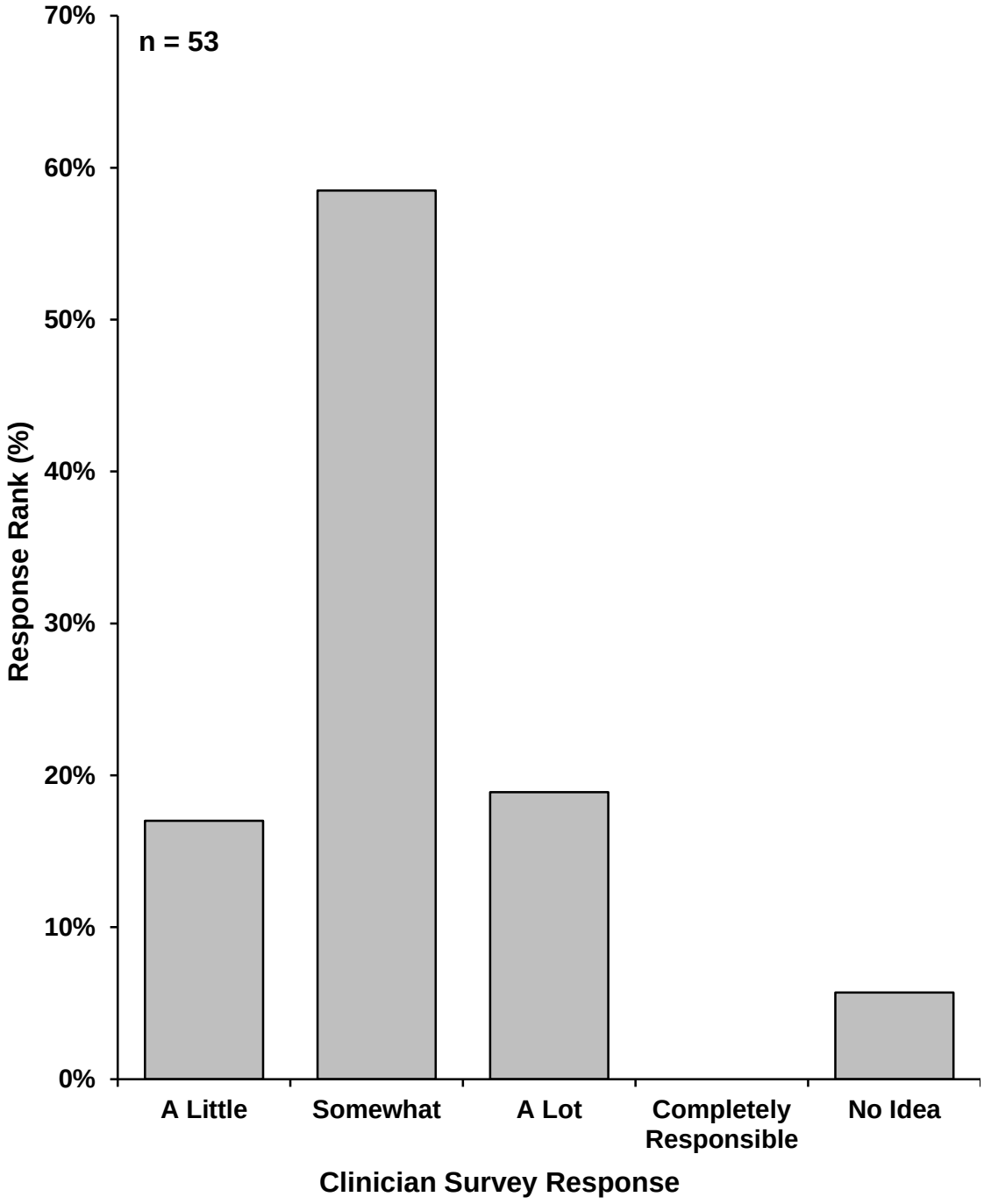

Figure 4.4. Percentage means of clinician responses on the significance of male age contribution on impaired semen and/or sperm cells demonstrated by the response rank to survey question number eleven.

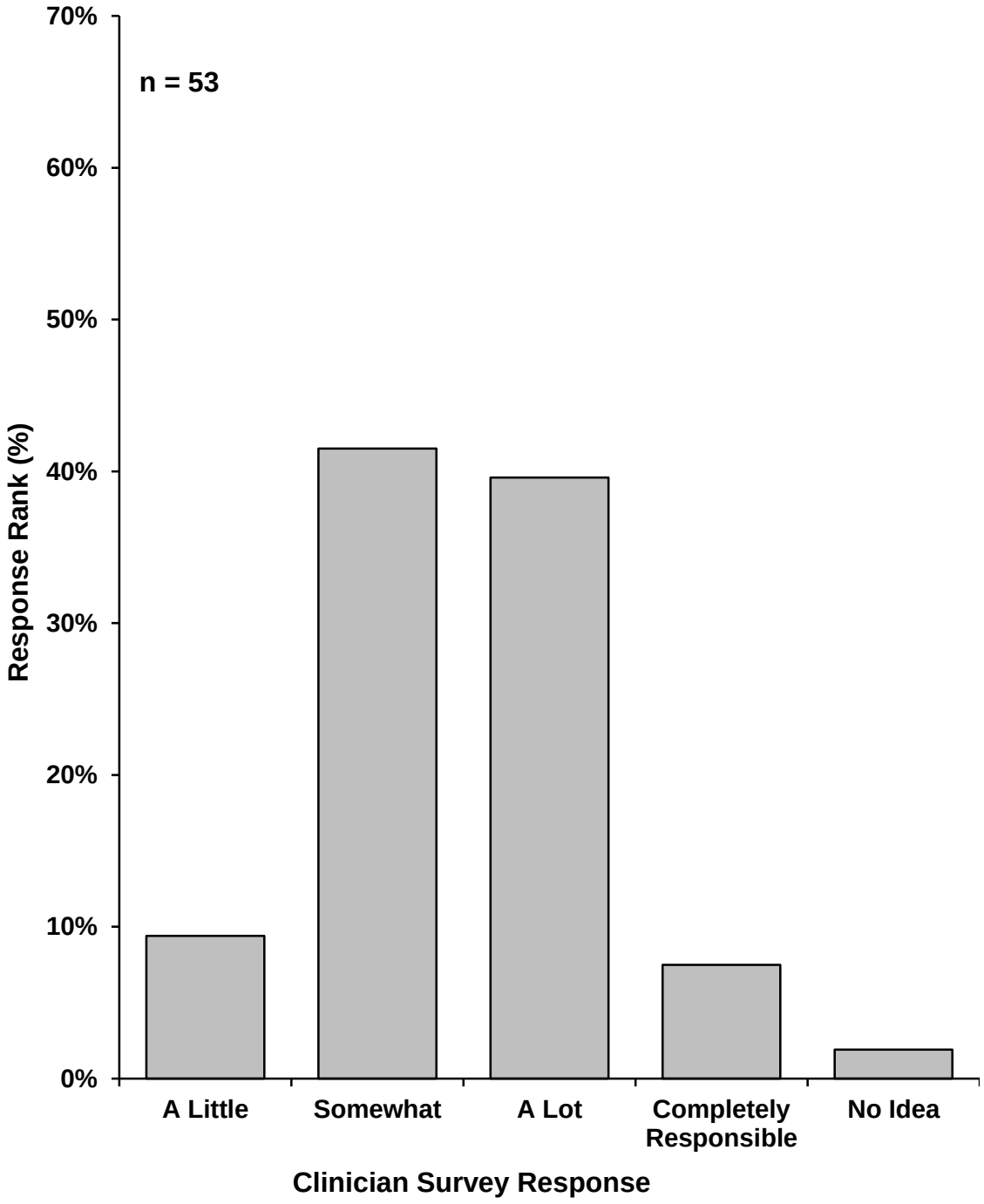

Figure 4.5. Percentage means of clinician responses on the significance of genetic/epigenetic changes in sperm DNA on impaired semen and/or sperm cells demonstrated by the response ranks for survey question number twelve.

Male exposure and environmental factors were thought to have a lot of significance on impaired semen and/or sperm cells as reported by 58.5% of clinicians surveyed (Figure 4.6). Approximately, 32.0% of the participants responded that male exposure and environmental factors were believed to have somewhat of a significant effect on normal sperm production. Lastly, 5.7% of the remaining opinion results demonstrated the belief that these factors had little significance on impaired semen samples and 3.8% of clinicians reported having no idea.

Considerably, when asked if access to more male patient lifestyle information would enable the clinician to provide better care, 53.9% of survey participants answered yes, 19.2% stated no, and 26.9% were undecided (Appendix F.8). Survey participants also responded to the need of a better clinical communication system for access to male records with 53.8% professional agreement, 42.3% thought the current system was sufficient, and 3.9% were undecided about the need for a better clinical communication system (Appendix F.9).

Objective 1

To describe the level of importance of male infertility variables that clinicians report most commonly observed in their professional experiences in comparison to the retrospective male response data collected on those same male infertility variables.

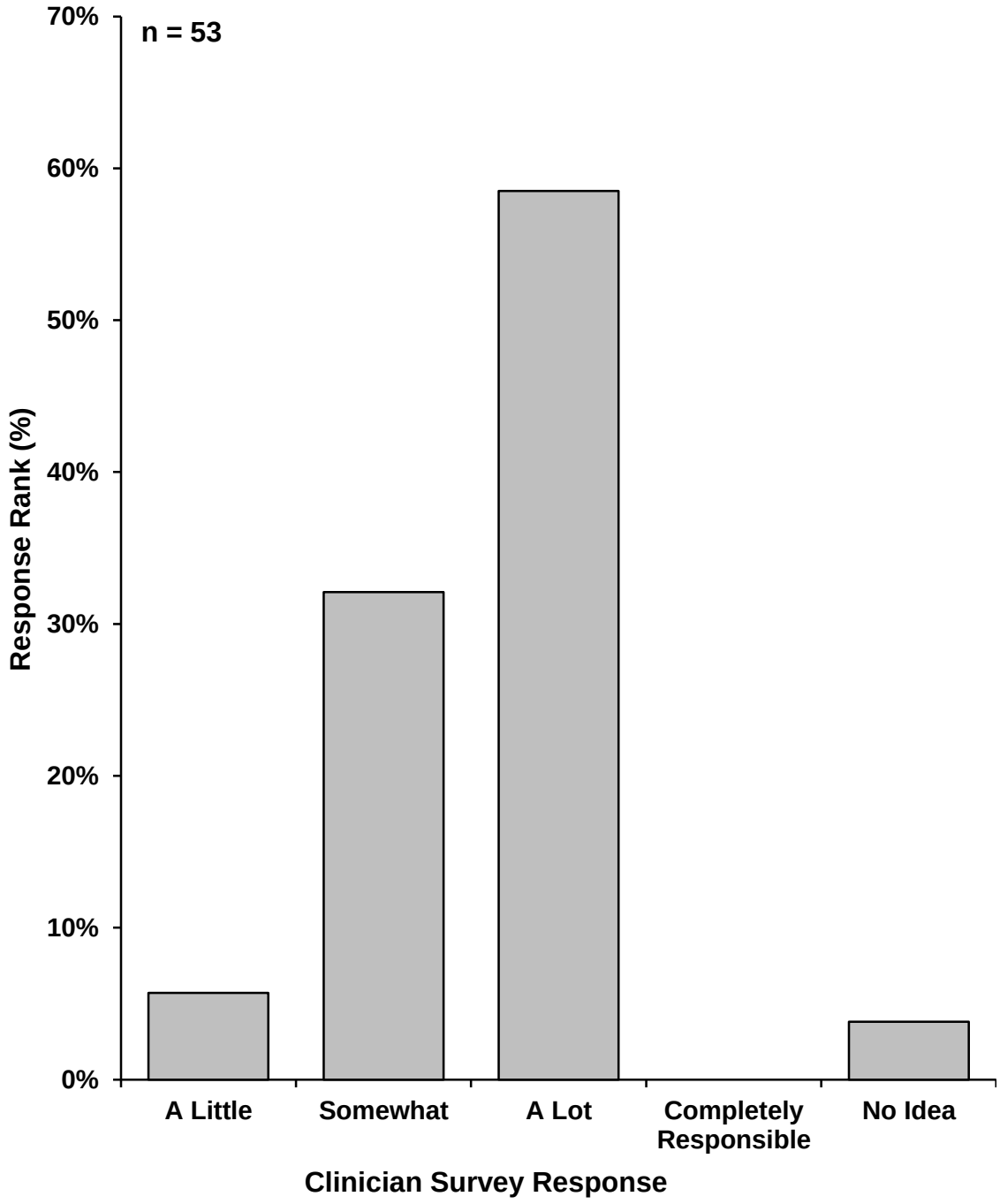

Figure 4.6. Percentage means of clinician responses on the significance of male exposure and environmental factor(s) contributed to sperm impairments as cells demonstrated by the response ranks for survey question number twenty.

The most commonly seen descriptive variables that clinical professionals reported in connection with male infertility were infertility length with current partner at 70.8%, age at 45.8%, BMI at 29.2%, and weight at 16.7% (Table 4.3). Height was added to the table because of its response rate in the retrospective study. Seventy percent of male infertility patients evaluated in the retrospective study reported data on height. Interestingly, only 2.1% of the clinicians surveyed listed height as an important descriptive variable. However, BMI was ranked third by survey responders, at 29.2%, among common variables associated with male infertility. Therefore, one would assume that height should be just as important as weight since BMI can be calculated if male patient records contain data on both.

Table 4.3. Percentages of the response rank of clinicians on the importance of male infertility variable and the response rate of retrospective study male infertility patients.

Male Factor Variable	**Survey Response Rank %**	**Retrospective Response Rate %**
	n = 53	**n = 83**
ILCP	70.8%	59.0%
Age	45.8%	98.0%
BMI	29.2%	60.0%
Weight	16.7%	65.0%
Height*	2.1%	71.0%

*Height was included because of retrospective response rate percentage.

As mentioned above, ILCP was the most commonly reported variable by survey participants at 70.8%. Retrospective data response rate for ILCP was lower at 59.0%. However, the retrospective percentage needs to be considered with caution, since some of the ILCP data was obtained from female partner charts in order to have more data samples. By having done this the researcher may have suppressed a larger difference that is not being expressed.

The most important semen characteristics that clinical professionals reported in correlation to male infertility was sperm count at 84.6%, sperm motility at 76.9%, sperm morphology at 75.0%, progressive motility at 46.2%, and volume at 17.3%. Retrospective response rates were considered to be available one hundred percent of the time (Table 4.4).

Table 4.4. Percentages of the response rank of clinicians on the importance of semen evaluation characteristics and the response rate of retrospective study male infertility patients.

Variable	Survey Response Rank %	Retrospective Response Rate %
	n = 52	n = 104
Sperm Count/Concentration	84.6%	100%
Sperm Motility	76.9%	100%
Sperm Morphology	75.0%	100%*
Progressive Motility	46.2%	100%**
Volume	17.3%	100%

**n = 100
*n = 90

It should be noted that the semen sample data listed for the retrospective response rate did not come from patient responses. Semen evaluations are routinely recorded in the male infertility patients' medical charts by their clinicians. Thus, with the exception of a few characteristics that are not evaluated after a sample is frozen and thawed, semen data should be routinely available for male infertility patients.

Data based on urology variables usually comes from the male patients' personal responses or medical records from a prior urological evaluation. In the clinician survey study, the most commonly seen urological variables reported in correlation to male infertility were vasectomy at 77.8%, hormone treatment at 60.0%, surgery to testicles at 33.3%, undescended testicles at 33.3%, and impotence at 28.9% (Table 4.5).

In the retrospective study, the researcher observed a number of missing data points for these specific variables. When evaluating impotence, the response rate was 54.0% of male patients in the retrospective study and the clinicians surveyed rated it lowest in importance. However, it was noted by the researcher that 15.0% of the retrospective patients had specifically skipped the same question dealing with impotence while completing all other remaining questions on the evaluation. Table 4.5 demonstrates the retrospective response rates as they compared to the clinicians' survey opinions. The retrospective results demonstrated a male patient completion response rate for vasectomy at 66.0%, for hormone treatment 61.0%, for surgery to testicle(s) 63.0%, and for undescended testicle(s) 63.0%.

Table 4.5. Urological male infertility variables of importance. Survey percentages describe response rank and retrospective percentages describe response rate.

Variable	Survey Response Rank %	Retrospective Response Rate %
	n = 48	n = 83
Vasectomy	77.8%	66.0%
Hormone Treatment	60.0%	61.0%
Surgery to Testicle(s)	33.3%	63.0%
Undescended Testicle(s)	33.3%	63.0%
Impotence	28.9%	54.0%

The most commonly seen environmental exposure variables that clinical professionals reported in correlation to male infertility were smoking at 74.5%, steroids for body building at 70.6%, recreational drug use at 58.8%, exposure to chemicals at 51.0%, recent high fever at 39.2%, and alcohol use at 39.2% (Table 4.6).

Again the male patient retrospective response rate was listed in Table 4.6 to be viewed in relation to the survey responses. Smoking resulted in the largest percent of retrospective data acquired at 66.0%; followed by steroids for body building at 62.0%, recreational drug use at 61.0%, exposure to chemicals 60.0%, recent high fever at 61.0%, and alcohol use at 59.1%.

Table 4.6. Environmental exposure male infertility variables of importance. Survey percentages describe response rank and retrospective percentages describe response rate.

Variable	Survey Response Rank %	Retrospective Response Rate %
	n = 52	n = 83
Smoking	74.5%	66.0%
Steroids for Body Building	70.6%	62.0%
Recreational Drug Use	58.8%	61.0%
Exposure to Chemicals	51.0%	60.0%
Recent High Fever	39.2%	61.0%
Alcoholic Use	39.2%	59.0%

Survey results revealed that the most commonly seen medical variables encountered by clinicians in correlation to male infertility were medication use at 84.8%, recent illness/infection at 50.0%, BMI at 37.0%, and birth defects at 21.7% (Table 4.7). Results for the response rate of the retrospective study showed 14.0% of the sample reporting medication use for high blood pressure. This was the highest frequency of type of medication retrospectively collected from male patient records. The complete percentage of patient data for medication use was 60.2%. In addition, 62.7% reported a recent illness and 65.1% had available BMI rates (Table 4.7).

Table 4.7. Medical male infertility variables of importance. Survey percentages describe response rank and retro percentages describe response rate.

Variable	Survey Response Rank %	Retrospective Response Rate %
	n = 52	n = 83
Medication Use	84.8%	60.2%
Recent Illness/Infection	50.0%	62.7%
BMI	37.0%	65.1%
Birth Defects	21.7%	N/A

2. To describe the relationship between the amounts of completed male medical records available from the retrospective analysis of data in comparison with the amount of completed male medical records reported as seen by clinicians and measured by an anonymous national online survey.

Clinician survey participants described that 39.1% of their charts were less than 25.0% completed, 34.8% of their charts were 25.0 to 50.0% completed, and 26.1% of male chart data was considered greater than 75.0% completed. Results of male patient chart completion evaluated in the retrospective study reported that 45.0% were less than 25.0% completed, 23.0% was 25.0 to 50.0% completed, and 32.0% were recorded as greater than 75.0% complete (Figure 4.7). These results are consistent with the observations of a number of current research studies. In a 2011 study, Billari et al. reported that survey response rates on reproductive age varied from 46.0 to 73.0%.

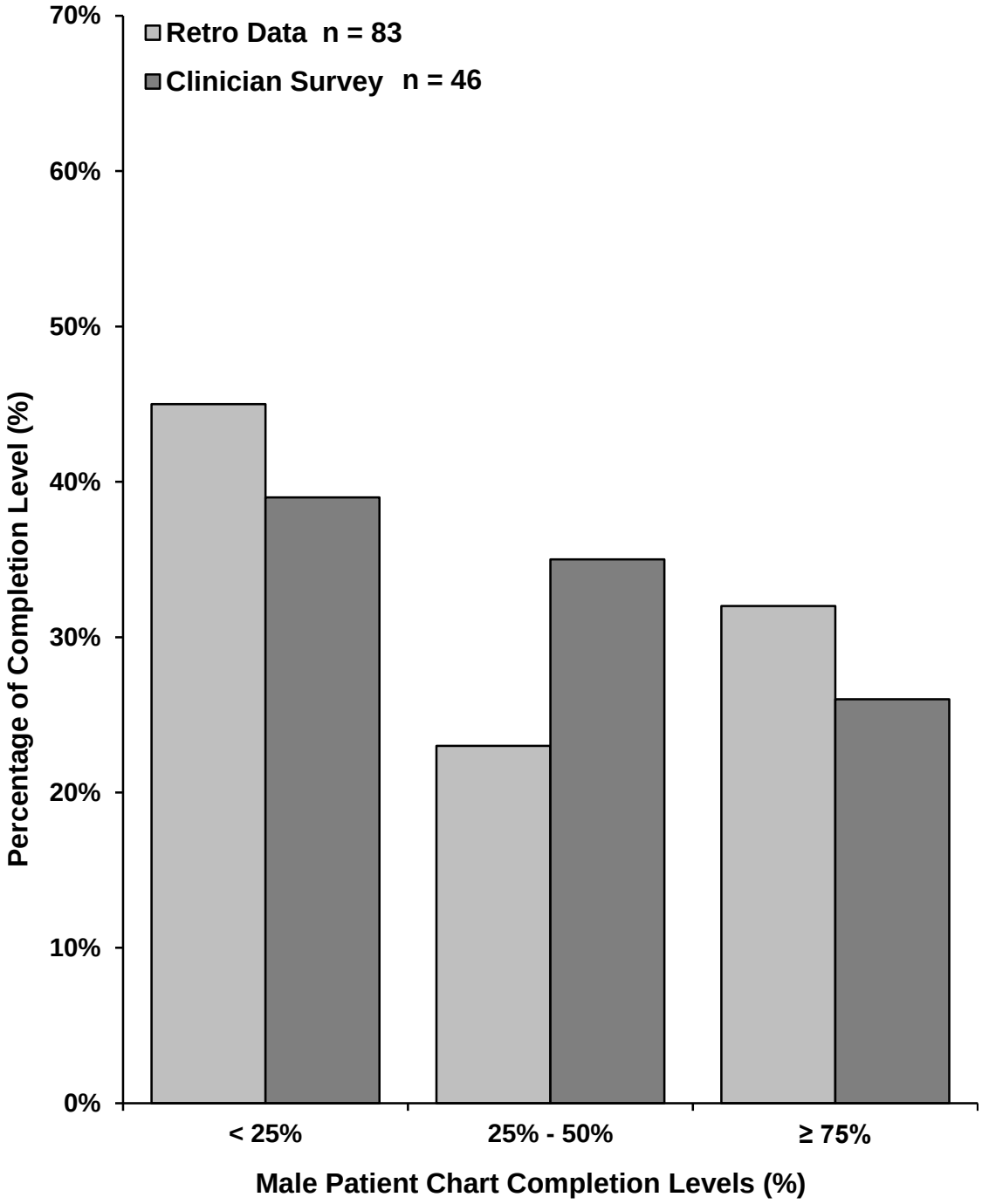

Figure 4.7. Percentage of retrospective study male patient chart completion levels (%) compared with percentage of male patient chart completion levels (%) as observed and reported from clinicians surveyed.

3. Clinicians who participated in the voluntary male infertility survey will demonstrate a negative correlation among their opinions of infertility topics such as; data availability and gender based ethical treatment of patients.

Results analyzed from a Cramer's V correlation coefficient found no significant relationship between gender and occupation with an r value, r = .326 (Table 4.8 and 4.9).

Table 4.8. Frequency distribution of the survey participants' gender and occupation presented in a Cramer's V correlation coefficient contingency table.

Gender*Occupation Contingency Table

	Occupation						
Gender	Repro Endo	Repro Phys	Embryologist	Andrologist	Urologist	Repro Tech	Total
Male	2	9	12	3	1	0	27
Female	1	4	18	2	0	1	26
Total	3	13	30	5	1	1	53

Table 4.9. Cramer's V correlation coefficient exhibits a non-significant relationship between gender and occupation.

Cramer's V Correlation Coefficient		Value	Approx. Sig.
Nominal by Nominal	Phi	.326	.343
	Cramer's V	.326	.343
N of Valid Cases		53	

Using a Cramer's V correlation, there was no significant correlation found between occupation and access to any of the data variables; descriptive at r = .351 (Appendix F.10a, F.10b), semen at r = .235 (Appendix F.11a, F.11b), urological at r = .317 (Appendix F.12a, F.12b), exposure at r = .251 (Appendix F.13a, F.13b), or medical data r = .306 (Appendix F.14a, F.14b). The results of this analysis were a bit surprising and contradictory to the participants' response of needing more data. Although, the researcher did not come across any published research studies based on infertility patient record availability, personal observation in the industry has indicated a difference.

The expected results for this correlation were that occupation status would have a significant relationship when compared with access to patient data. The results were expected to more closely reflect the respondents' opinion of needing a better communication system, which 53.8% of the professionals agreed upon.

Using a Cramer's V correlation coefficient there was no significant correlation found between gender and the need for a better clinical communication system with a value of r = .284 (Table 4.10 and 4.11). Furthermore, a non-significant relationship was identified when Cramer's V was used to analyze occupation and the need for a better communication system (Table 4.12 and 4.13).

Table 4.10. Frequency distribution of survey participants' gender and opinions on the need for a better communication system presented in a Cramer's V correlation coefficient contingency table.

Gender*Clinical Communication System Contingency Table

	Clinical Communication System			
Gender	Current System Sufficient	Need Better System	Undecided	Total
Male	8	16	2	26
Female	14	12	0	26
Total	22	28	2	52

Table 4.11. Cramer's V correlation coefficient exhibits a non-significant relationship between gender and the need for a better clinical communication system.

Cramer's V Correlation Coefficient		Value	Approx. Sig.
Nominal by Nominal	Phi	.284	.122
	Cramer's V	.284	.122
N of Valid Cases		52	

Table 4.12. Cramer's V correlation coefficient exhibits a non-significant relationship between occupation and the need for a better clinical communication system.

Occupation*Clinical Communication System Contingency Table

	Clinical Communication System			
Occupation	Current System Sufficient	Need Better System	Undecided	Total
Repro Endo	2	1	0	3
Repro Phys	3	9	1	13
Embryologist	14	15	1	30
Andrologist	3	1	0	4
Urologist	0	1	0	1
Repro Tech	0	1	0	1
Total	22	28	2	52

Table 4.13. Cramer's V correlation coefficient exhibits a non-significant relationship between occupation and the need for a better clinical communication system.

Cramer's V Correlation Coefficient		Value	Approx. Sig.
Nominal by Nominal	Phi	.359	.752
	Cramer's V	.254	.752
N of Valid Cases		52	

As expected, when addressing the ethical topic of male infertility patient cutoff age responses varied among the clinicians. In reference to the loose establishment of female cutoff ages, survey participants were questioned on their experiences with male cutoff ages. Regarding their own personal clinics, 94.2% of survey participants responded that they did not enforce a male cutoff age for infertility treatment. Furthermore, responses demonstrated that only 5.8% of the professionals implemented a clinical male cutoff age as shown in Figure 4.8.

If participants responded yes to the previous question they were asked to specify a male cutoff age. With a 10.0% response rate, clinicians reported inconsistent guidelines on the need to set an age. In addition, the actual male cutoff age currently enforced by their clinic was also highly variable. Responses ranged from a suggested male age of 40 years old for semen donors only, to a 60 and/or 65 year old male patient age treatment limit. In addition, 2.0% of those that responded explained that male patients over 40 years of age at their clinic were only counseled on the increased risks of advanced male reproductive age.

Although these limited responses were not analyzed for significant differences, it is important to note the response variation that existed among such a small population. Since it is believed that the study sample is a normal representation of the infertility industry population, the researcher found that these results were reasonably generalizable to the industry as a whole. The previous review of literature supported these results, as well, by citing examples of inconsistent opinions and practices among clinicians in the fertility industry.

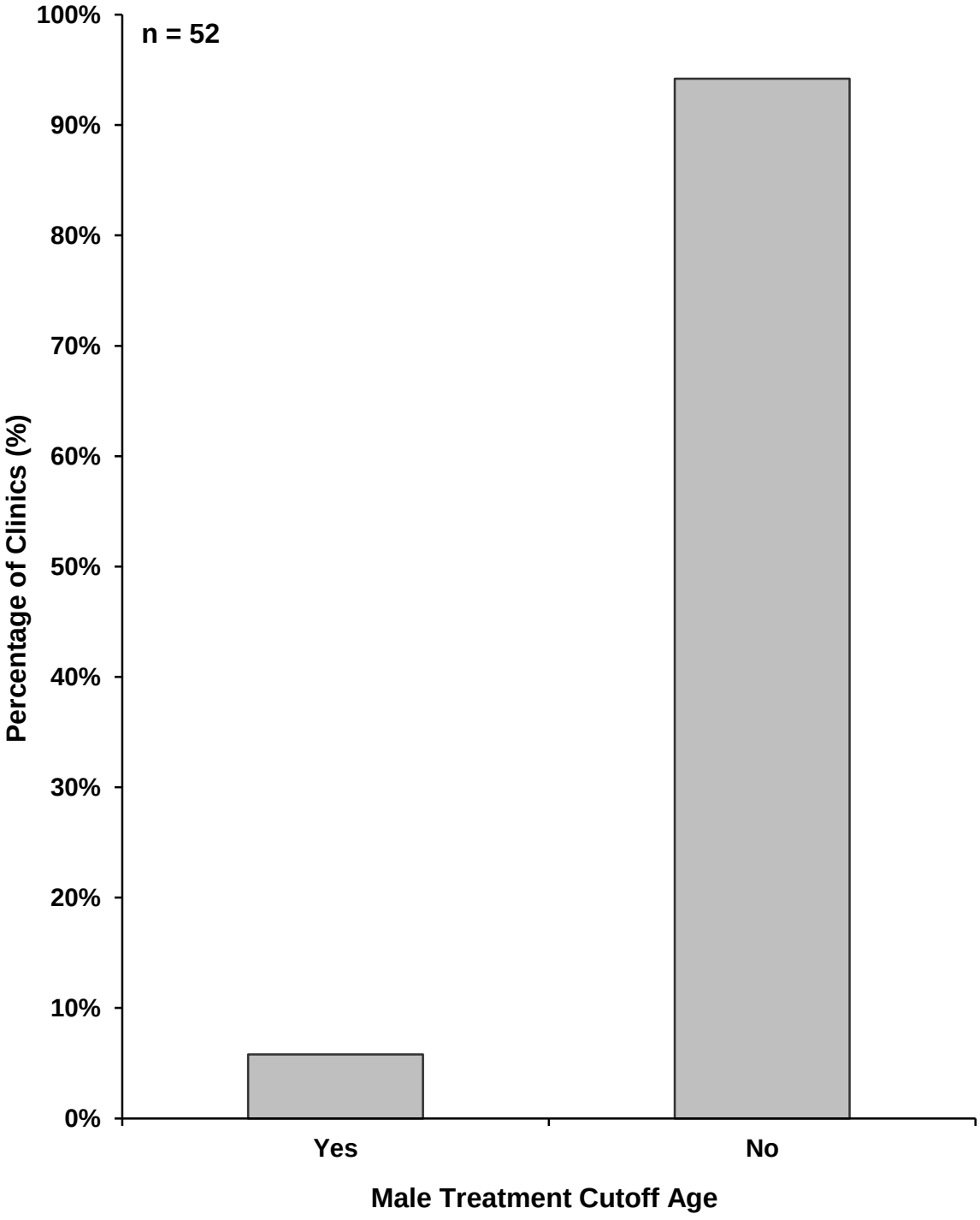

Figure 4.8. Percentage of clinics that enforce a male infertility treatment cutoff age as reported by clinicians surveyed.

When clinicians were asked their ethical opinion of providing fertility treatment for males after a certain age, 35.0% responded it was unethical and 64.0% responded it was not unethical (Table 4.14). Using a Phi correlation coefficient, a significant relationship was not found among clinician gender and their opinion to enforce a male treatment cutoff age, with an r value of r = .162 (Table 4.15).

Table 4.14. Frequency distribution of survey participants' gender and opinions on unethical treatment of advanced age male patients presented in a Phi correlation coefficient contingency table.

Gender*Unethical Advanced Age Male Treatment Contingency Table

		Gender		
Unethical Advanced Age Male Treatment		Female	Male	Total
	No	19	15	34
	Yes	7	11	18
Total		26	26	52

Table 4.15. Phi correlation coefficient exhibits a non-significant relationship between gender and opinions on unethical treatment of advanced age male patients.

Phi Correlation Coefficient		Value	Approx. Sig.
Nominal by Nominal	Phi	.162	.244
	Cramer's V	.162	.244
N of Valid Cases		52	

As previously stated, male infertility is a considerably understudied area of research. In alignment with this circumstance and the low male response rates reported in both studies; the researcher wanted to evaluate clinicians' opinions in regards to the ethical question of adequate information being provided. As a whole, survey participants responded to the issue as expected (Table 4.16). An impressive 63.0% of clinicians acknowledged that the industry was not providing enough information to patients on the risks associated with advanced male age reproductive treatments (Figure 4.9). As demonstrated in Table 4.17, a Cramer's V analysis, reported a p value of r = .120. The results from this correlation established that clinician gender did not influence clinicians' opinions on adequate male information being provided to patients.

By comparing these two variables the researcher wanted to determine if a relationship existed between clinician gender and the response to a gender based question on ethical treatment. The researcher expected the results to show that this variable was, in fact, a significant positive or negative factor, due to the gender sensitivity of this question.

To identify the possible existence of a relationship between occupation and adequate male information provided, the researcher again used a Cramer's V correlation coefficient. With an established p value of r = .310, survey results demonstrated that clinician occupation did not influence the clinicians' opinions of adequate male risk factor information provided (Figure 4.18 and 4.19).

Table 4.16. Frequency distribution of survey participants' gender and opinions on adequate male data provided presented in a Cramer's V correlation coefficient contingency table.

Gender*Adequate Male Data Provided Contingency Table

	Adequate Male Data Provided			
Gender	No	Yes	Undecided	Total
Male	15	7	4	26
Female	18	5	3	26
Total	33	12	7	52

Table 4.17. Cramer's V correlation coefficient exhibits a non-significant relationship between gender and adequate male data provided.

Cramer's V Correlation Coefficient		Value	Approx. Sig.
Nominal by Nominal	Phi	.120	.688
	Cramer's V	.120	.688
N of Valid Cases		52	

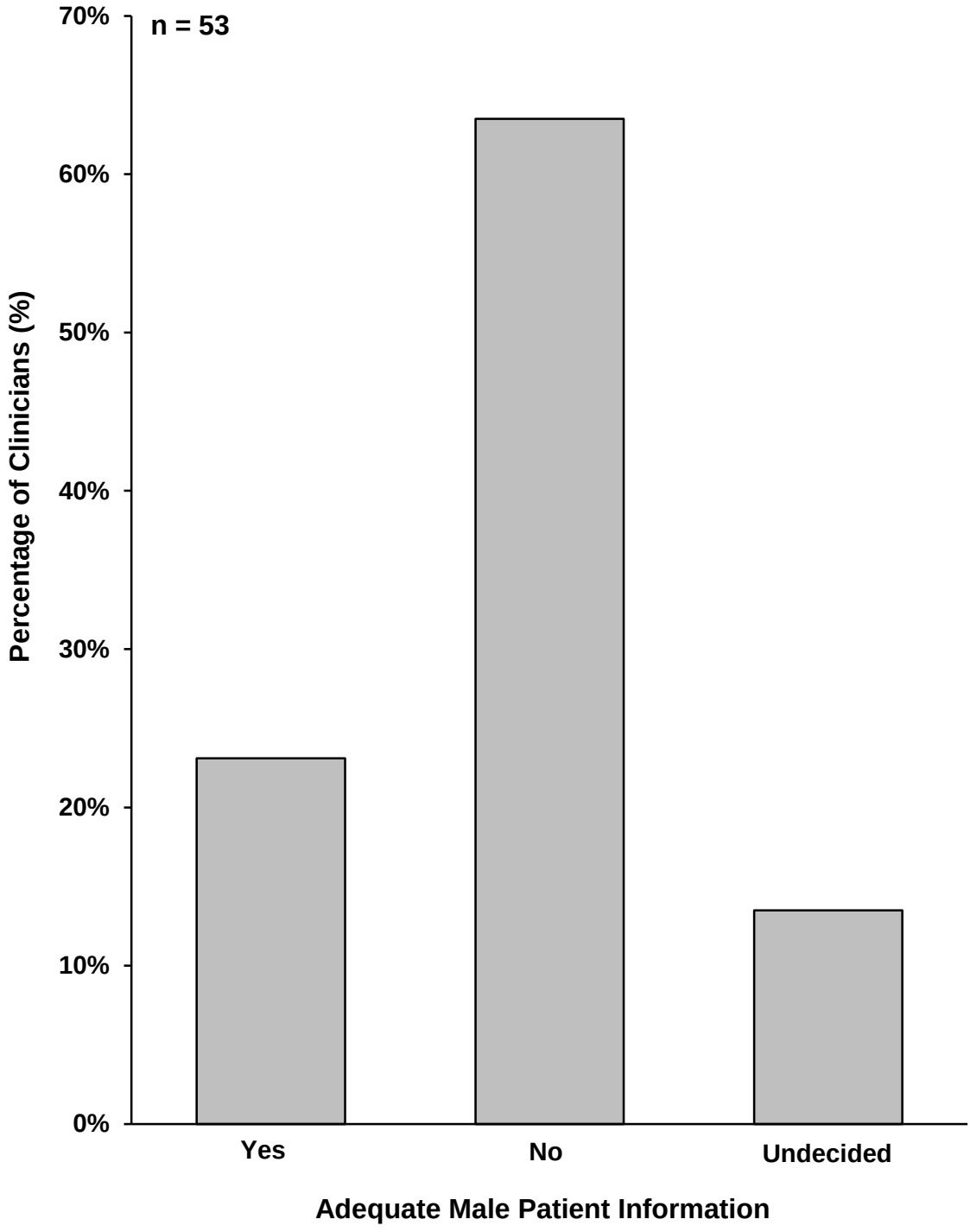

Figure 4.9. Percentages of clinicians' survey opinions on adequately providing substantial information to patients on advanced age male reproductive risks.

Table 4.18. Frequency distribution of survey participants' occupation and adequate male data provided presented in a Cramer's V correlation coefficient contingency table.

Occupation*Adequate Male Data Contingency Table

	Adequate Male Information Provided			
Occupation	No	Yes	Undecided	Total
Repro Endo	2	1	0	3
Repro Phys	9	4	0	13
Embyologist	18	5	7	30
Andrologist	3	1	0	4
Urologist	0	1	0	1
Reproductive Tech	1	0	0	1
Total	33	12	7	52

Table 4.19. Cramer's V correlation coefficient exhibits a non-significant relationship between occupation and adequate male data provided.

Cramer's V Correlation Coefficient		Value	Approx. Sig.
Nominal by Nominal	Phi	.438	.441
	Cramer's V	.310	.441
N of Valid Cases		52	

Discussion

The main purpose of including the clinician survey into this research project was to gain a more realistic grasp on controversial issues in the male infertility industry. Although, not much statistical significance can be taken from this design, it gives the reader an idea of what is happening in the industry daily. In addition, it highlights new areas of ideas on male infertility research. The key is to connect what the clinicians are currently observing and the areas that need to be researched.

The goal of this objective was to view the two rates, clinician response and retrospective male patient data, side by side and evaluate for any possible relationships. Survey percentages describe the response rank of clinicians and retrospective percentages describe the response rate of male infertility patients. Further research can be developed based upon variables that clinicians think acknowledge as important to male reproductive success. Those variables can also be compared with their availability from male patient responses to clinical questionnaires, to assess question sensitivity.

While only 45.8% of the survey participants regarded male age as an infertility indicator; the previous chapter study reported data that found a significant correlation between male age and biochemical pregnancy rates. Retrospective data also revealed a significant correlation between male age and IVF pregnancy rates. However, it is important to observe from the survey results that clinicians are not focused only on male age as an infertility factor. The

results suggested that clinicians are observing additional male infertility and environmental factors; just as current research has begun to demonstrate.

Participating clinicians reported that the variable of highest average of male infertility factor importance observed from their perspective was infertility length with current partner. However, the results from the retrospective study of male patients were so variable that it provided no significance. These contrasting results may be the expression of the difference in study design. A possible suggestion is that clinicians obtain altered data from face to face patient care when compared with the patient responses gathered from a questionnaire.
It is safe to assume that the missing retrospective data on ILCP in combination with the already small sample size was a limiting factor in the previous study. This may prove to be an important difference when comparing the results of the two studies. Substantial research studies in this area should be designed around the most effective methods of gathering patient data on infertility length.

Additionally, this may be an area where patients need access to more fertility information. In their 2007 paper, Robinson and Ellis reported that one of the probable causes for failure to conceive appeared to be mistiming of intercourse. One way for clinicians to address this issue would be to provide patients with increased knowledge on fertilization and ovulation in an effort to reduce infertility length in some cases.

When comparing semen evaluation characteristics, the results of the two studies expressed different main variables. Results from the retrospective study reported semen volume and progressive motility as negatively correlated to male

age. These age related variables are commonly reported among semen evaluation research studies, as recognized in the review of literature. However, only 17.3% of survey participants reported volume as an important semen characteristic and the second to lowest importance rating was for progressive motility (46.2%).

In the retrospective study, smoking was reported to have multiple significant correlations with semen characteristics. Similarly, survey participants reported smoking as the top variable affecting male fertility in their experience. A number of research studies, such as Linsten et al. (2005), reported both smoking and overweight to unfavorably affect male reproductive success after IVF cycles. Linsten and colleagues (2005) went on to report that the negative impact of smoking on the live birth rate in IVF treatment is comparable to an increase in female age >10 years.

Vasectomy was listed as the most common seen urological factor in relation to male infertility. However, out of 83 responses in the retrospective study only six percent of male patients reported having a vasectomy. Two percent reported having had hormone treatment as compared with 60.0% of clinicians reporting its importance. One percent reported having surgery to testicles as compared with 33.0% percent of clinicians reporting its importance. One percent of the male patients reported having undescended testicle as compared with 33.3% and 8.0% percent reported impotence as compared with 28.9% of clinicians reporting its importance.

When participating clinicians were asked about the role DNA fragmentation plays in male infertility, the majority, 33.0% reported having no idea. In addition, DNA fragmentation represented one of the lowest semen variables that clinicians reported testing. At 30.2%, less than half of the clinics actually reported to testing for DNA fragmentation. Due to limited research on DNA fragmentation, the opinions of clinicians may be reflecting the lack of knowledge on alternative semen characteristics effecting male fertility. Further analytical studies are needed to identify the relationship between DNA, semen characteristics, and biochemical pregnancy rates.

Not all infertility researchers agree on the same ‘normal’ characteristics for a semen analysis. The review of literature explained how most of the semen variables tested for fertility are controversial. In the current survey clinicians reported that semen quality is a high predictor of reproductive success. However, 66.0% of the group reported that a better diagnostics test is needed for semen analyses. Sousa et al. (2011) are among some of the researchers that have demonstrated advanced techniques to evaluate semen samples. Yet, a subpopulation containing only fertile sperm has never been isolated. The researchers explained that this was mainly due to the fact that we still cannot completely describe what makes a competent spermatozoon (Sousa, et al., 2011).

Although the researcher expected to see a difference in reporting based on gender; the results of the current survey showed no correlation between male and female clinicians as they responded to specific gender based questions.

These results were in agreement with a 2011 human fertility age limit study published by Billari and colleagues. When evaluating participants, gender of the respondent was not a statistically different indicator in the perception of female or male age deadlines (Billari et al., 2001). Some researchers have suggested that as society advances, gender equality plays a role in the notion of fertility equality.

The majority of clinicians surveyed were in agreement that more male information would be beneficial in the treatment process. Study results demonstrated similar percentages between the experiences of clinicians and the amount of retrospective data reported when analyzing the issue of missing male data. A 2014 CDC consensus illustrated that the percentage of male data reported to analyze was much less than the amount of female data available (Center for Disease Control and Prevention, 2014).

A likely factor contributing to insufficient reporting is the stigma associated with certain male medical conditions. In a 2003 review, Dudgeon and Inhorn concluded that for men, infertility is a potentially humiliating and emasculating stigma that had a more profound adverse impact on men than the same diagnosis did for women. The group argued that in contemporary Western societies, stereotyped masculinity denies vulnerability, promotes an appearance of toughness and emotional control, minimizes the need for assistance from others, and suggests a preoccupation with sex, which virtually leads men to view male infertility the same as male impotence (Dudgeon and Inhorn, 2003).

Another proposed reason for so much missing data is the male patients' lack of fertility knowledge on the importance of the question. For example, some

clinics in the industry provide professional consultations to advanced age female patients, while similar programs are not nearly as often provided for males. Additionally, outside of the fertility world, reproductive problems and prenatal care are generally associated with human females.

This raises the question, is the fertility industry providing adequate information on male risk factors, such as advanced age and exposure. In addition, are patients aware of the ethical issues associated with advanced age fertility treatments? The response data from this survey suggests that 63.5% of the professionals surveyed do not think adequate male infertility information is being addressed with infertility couples. Although, this was not statistically analyzed for significance, it can be viewed as a current evaluation describing the professional practices in today's human infertility clinics.

There are fertility clinicians within the industry who have a strong opinion about paternal age and others that do not. Ultimately, it is up to the clinic and the patient to decide upon treatment. Currently, few federal laws interfere with U.S. citizens becoming parents. In the U.S. there is not one federal law in place that enforces an age limit on becoming a parent. Therefore, it is not required but some clinics practice female cutoff ages, while very few have male cutoff ages.

Surveying the views of clinicians in the current study provided an example of industry practices. Ninety-four percent of professionals responded that they do not have a male cutoff age. However, when asked if it was unethical to provide fertility treatment for males after a certain age the percentage of clinicians who responded yes rose to 35.0%, while 65.0% responded no. More research on

advanced male age factors will help to provide additional needed clarity to the issue of ethical patient treatment.

Due to the exploratory nature of the study, no significant conclusions were drawn from the comparison of relationships among this data. Although the sample size of the clinician survey study was small, the researcher felt that the sample population was normally distributed. Therefore, opinions reported can be generalized as comparable to those of clinicians throughout the industry. Additionally, since there are so few industry standards enforced, gaining any information on the current practices will be beneficial. The current study results will contribute to the foundation of future male infertility research. Feedback from the clinicians provided researchable information on the amount of male risk factors and lifestyle factors currently being addressed with infertility couples. By reporting patient data and performing new studies like the current survey, valuable exposure can be added to the area of male infertility research.

CHAPTER V: CONCLUSION

Summary

The focus of this research was the evaluation of male reproductive success in regard to age, as well as environmental lifestyle exposures. Biochemical pregnancy rate and semen parameters were the specific concentrations of the study. In 2010, Sartorius and Nieschlag reported that lower fertility rates and pregnancy-associated complications should not only be associated with advanced maternal age but also with increased paternal.

Much controversy about male infertility and the influential factors can be found among the current collection of literature. The present study was designed as a two part evaluation. A retrospective collection of male data from a private fertility clinic was combined with a national survey of infertility clinicians. The strategy of using these two approaches was to gather information about male infertility from different angles. The retrospective study served as an analysis of patient data, while the clinician survey functioned as an exploration of opinions within the industry. Combining the results of the two studies will provide investigators with additional data to determine relevant areas of research to investigate.

In 2011, Billari and colleagues pointed out that in light of the recent increase in fertility at advanced ages, it is important to understand both the factors that drive this increase and the factors that limit this increase. Biology and reproductive technology have set ultimate limits on fertility, especially for females (Billari et al., 2011). Male limits, on the other hand, are much less defined. Recent research has shown that men do in fact have biological clocks affecting

hormone levels, fertility, and sperm quality (Lambert et al., 2006; Lewis et al., 2006). Although, opposed to females, male reproductive function alters slowly over a period of years, albeit with age dependent alterations (Sartorius and Nieschlag, 2010).

Increasing research on the topic of male fertility brings investigators closer to defining the factors that improve male reproductive success and the factors that hinder male reproductive success. With each study, the role of male infertility becomes more clarified in its recognition as a dual contributor to a couple's reproductive success.

Implications, Recommendations, Limitations

Retrospective Study

Conclusion 1

It was concluded that advanced age males who participate in clinical ART treatment cycles at a private human fertility clinic will have lower biochemical pregnancy rates and lower IVF pregnancy rates. These results are supported by a number of research studies. In a 2004 study, Kühnert and Nieschlag showed increasing evidence that advanced paternal age is associated with changes in reproductive functions on different levels; semen production, fertility, and pregnancy outcome to name a few. These results are also in agreement with the 2006 study performed by de La Rochenbrochard and colleagues where the group demonstrated that advanced paternal age was associated with lower IVF rates.

It was also concluded that advanced age males who participate in clinical ART treatment cycles at a private human fertility clinic will have decreased seminal volume and reduced progressive motility. Similarly, Rolf and colleagues (1996) demonstrated that seminal volume and seminal fructose concentration decreased with age, possibly due to a seminal vesicle insufficiency, since the seminal vesicle contributes most to ejaculate volume. In 2006, Gagnon and de Lamirande explained that factors leading to decreased sperm motility could be found in altered functions of post-testicular glands such as the prostate and, more probable, the epididymis, as the swimming ability of spermatozoa is acquired during epididymal transit and motility is dependent on dilution into seminal plasma.

Additionally, age-dependent alterations of the epididymis might lead to disturbed mitochondrial functioning. An important part of epididymal sperm maturation is the activation of sperm mitochondria (Aitken et al., 2007). The mitochondria are essential for energy production and storage, which enables the sperm to remain motile. In 2011, Sousa et al. suggested that differences in sperm fertilization ability between ejaculates can be attributed to the number of sperm in the ejaculate with functioning mitochondria.

The current study results concluded that advanced age males who participate in clinical ART treatment cycles at a private human fertility clinic did not experience unsuccessful semen characteristics for concentration, motility, percent normal, and total motile sperm per specimen. These results are not in agreement with prior research data from the same fertility clinic. The clinic

previously concluded that sperm concentration, motility, and morphology decreased as male age increased. The difference between the current study and the previous clinical research was the sample size of male patients and the length of time male patients were evaluated. The current study contained a sample size of 104 male patients and evaluated cycles over a four year period. The previous research contained a sample size of 16,156 male patients and evaluated cycles over a nine year period.

Although no significant correlations were found when advanced male age was compared with the patient's response for difficulty with ejaculation, the results led the researcher to believe that there is a definite need for further studies in this area. It is believed that an abnormally high amount of male patients specifically skipped the two questions related to difficulty with erection and ejaculation. The amount missing data strongly suggests that this may be a sensitive question for males to answer. Additionally, it raises the question of the accuracy of the results in studies that included this variable.

Previous female studies have associated advanced age with decreased implantation rates and pregnancy rates (Yarali et al., 2010; Jiao et al., 2002; Ishii et al., 2012). In a 2010 study, Yarali and colleagues found that increased miscarriages as well as decreased implantation rate were mainly responsible for the poor performance of patients with advanced female age. Duran and colleagues reported that although they observed a decrease in semen volume, sperm motility, and fertilization rate with advanced male age, embryo quality, clinical pregnancy, implantation, miscarriage, and live birth rates were not

affected (Duran et al., 2010). The current study results demonstrated an altered outcome.

This study was different in that it evaluated pregnancy rates and not fertilization rates. It is important to point out that all of the embryos transferred in the retrospective study cycles were fertilized. The detrimental effects were expressed at implantation or at the early stages of pregnancy development. The current study results found that advanced male age affected semen volume and progressive motility, as well as, biochemical pregnancy rates. The study went a step further by not only reporting decreased semen quality with advanced male age but also reporting decreased pregnancy rates after embryo transfer with advanced male age.

A recommendation for further retrospective research would be to obtain a larger sample size. The same variables should be addressed, with the inclusion of additionally identified factors. However, retrospective studies should be performed independently for variables associated with embryo quality, semen characteristics, and pregnancy outcome. Another recommendation for further research would be to perform a prospective research study at a human fertility clinic, addressing the same variables. Again a larger sample size would be needed, but the advantage of having present access to the patients could clear up some of the problems of missing data.

Conclusion 2

It was concluded that those male patients who reported to smoke more than 5 to 10 cigarettes per day showed a decrease in sperm concentration and

progressive motility. Additionally, it was concluded that male patients who reported smoking were also associated with ejaculation difficulty. Previous studies that have examined environmental factors and paternal fertility have also demonstrated an association between cigarette smoke and sperm concentration.

In a 1992 review of semen quality studies conducted over a 50 year period, Carlsen et al. reported that mean sperm concentration worldwide fell by half. Giwercman and colleagues (1993) concluded that such a fast decline in semen quality was probably due to environmental, rather than genetic factors. The group suggested that in utero exposure to environmental oestrogens, pollution, and lifestyle exposures, including cigarette smoking were possible causes of this decline in quality (Giwercman et al., 1993). In an additional review, Vine (1996) revealed that smokers' sperm concentration was on average about 15.0% lower than that of non-smokers.

The current study results concluded that male patients who participate in clinical ART treatment cycles at a private human fertility clinic demonstrated no decline in biochemical pregnancy rate when reported to consume 1 to 5 drinks socially. However, it was concluded that male patient alcohol usage of 1 to 5 drinks socially caused a decline in semen volume as well as total motile sperm per specimen.

The results of male consumption of alcohol in association with decreased semen volume are in agreement with the results of a 2014 study performed by Jurewicz and colleagues. Previous reviews were also in agreement that higher

age, smoking, and alcohol consumption were risk factors for poor semen quality (Li et al., 2011; Sadeu et al., 2010).

Sartorius and Nieschlag (2010) claimed that although a contribution of environmental factors to the deterioration of human semen parameters in advancing age is readily accepted, solid evidence does not exist. Future studies should take this into consideration. Larger study samples should be a major goal in future research. Due to the amount of missing data and the variability of male factors, such as semen characteristics, small samples may not be as effective in exposing significant male infertility factors.

Conclusion 3

It was concluded that advanced age males who participate in clinical ART treatment cycles at a private human fertility clinic will not have lower reproductive success rates as male BMI levels increase. Conversely, Kort and colleagues (2006) previously reported an inverse relationship between BMI and the total number of normal-motile sperm cells per subject observed. Interestingly, the group of men with a BMI greater than 25 kg/m2 had fewer normal chromatin-intact motile sperm cells per ejaculate.

In the current study we evaluated for common semen characteristics and biochemical pregnancy. However, other studies have suggested that BMI affected all parts of male reproduction, the effect commonly seen in the developing embryo. In 2014, Simon and colleagues showed that increased sperm DNA damage adversely affected embryo quality starting at day 2 of early

embryonic development and continuing after embryo transfer. This resulted in reduced implantation rates and pregnancy outcome.

Rato and colleagues (2014) reported that testicular metabolic alterations induced by increased adipose tissue may also lead to mitochondrial dysfunction, which is closely associated to reactive oxygen species (ROS) overproduction, and oxidative stress. ROS easily targets spermatozoa DNA and lipids, contributing to decreased sperm quality (Rato et al., 2014).

Therefore, it can be assumed, that embryo production is compromised because of sperm fertilization from a male with a high BMI level. Additionally suggesting that if BMI plays a factor in decreased semen quality it is possibly seen through DNA damage. In 2004, Tesarik et al., described how the sperm activates its genome at the time of embryo genomic activation and this crucial step determines the development of the embryo until the blastocyst stage. This is quite possibly where the BMI factor presents itself.

The current study did not evaluate for DNA damage or embryo quality. However, when erectile dysfunction was re-evaluated because of the amount of intentionally skipped questions the researcher noticed a trend in increased male patient BMI levels. Further research is needed to perform correlation studies on the relationships of these variables. Using the same variables, with the addition of embryo development and DNA damage, further studies should evaluate variables from a larger sample size of male patients. In the meantime, to ensure maximum fertility potential, male patients should still be advised to reduce body weight.

Conclusion 4

It was concluded that advanced age males who participated in clinical ART treatment cycles at a private human fertility clinic will have a decline in the percent of normal sperm as infertility length with their current partner increases. These results are in agreement with previous studies which suggested that the older a man was, the longer it may take his partner to conceive, regardless of her age (Ford et al., 2000). More recent studies have emerged stating that underlying infertility and time to pregnancy is another risk factor for adverse pregnancy outcomes, independent of maternal age (Zhu et al., 2006).

Unlike the small sample size of the current study, Ford and colleagues (2000) found significant correlation among older men and ILCP by performing a large population study. The current sample size for ILCP was not only small but extremely variable. Therefore, these results are to be considered with caution. There is an extremely limited amount of research in this area. Further studies need to pursue larger sample sizes and more accurate data responses.

Limitations

In addition to the limitations of a retrospective study design, there may also be unidentified correlations due to the small sample size of the study. Missing data contributed to another limitation of the study, correlation analyses were carried out for sub-samples, further reducing the sample sizes. To try and lesson these affects the researcher incorporated statistical procedures that exhibited normal distribution and an absence of significant differences in means when compared to the national population. Another limitation was the design of

the study only looking at couples biochemical pregnancy rates and semen characteristics. Researchers say the findings may actually underestimate the effect of male age on infertility and future studies should also include embryo development and live birth rates.

The small size of the sample can be considered a limitation to the current study. Due, in part, to such a small study sample the results cannot be adequately compared to previous studies that claimed paternal occupation (Kenkel et al., 2001; Magnusdottir et al., 2005) and lifestyle factors (Jensen et al., 2004; Povey et al., 2012; Sallmén et al., 2006) have an impact on semen parameters.

Using only one clinic for the sample strengthened the internal validity of the study but it came with limitations of getting a large enough sample size. Larger samples are usually obtained by a retrospective survey over a number of years. In that instance, the available data, complete or incomplete is all that the researcher has available to record.

Another limitation may be the increased success rate of the clinic. However, the researcher took measures to make sure that the sample was not significantly different from the national population. When clinical samples were statically compared to national population means there was no difference found. Advanced success of the study clinic may act as another limitation by overcoming some of the subtleties of male infertility factors. Even though correlations were made, causal inferences could not because of the design of the study.

Additionally, the researcher extensively reviewed both patient medical records of the sample couple to obtain as much information as possible. For example, missing male ILCP was obtained from female partner records when available. This was not done often, but it does contribute to the hindrance of the true response rate of the male. In addition, there were a number of discrepancies among male data, such as; males that listed different ages in separate documents, males and females that listed different ILCP, males that changed height and weight from one document to another, and the number of questions skipped.

Finally, another limitation to consider would be the generalizability of the study sample. However, the research group felt that securing internal validity was of greater importance. In addition, the study clinic routinely provides treatment to a large variety of patients from a large and diverse area among the southwestern region of the U.S.

Clinician Survey Study

Objective 1 and 2

As anticipated, results of the clinician survey suggested areas of concern for further research on male variables and practices within the industry based on their experiences with male infertility patients. Specific male variables of importance were identified from the opinion results of clinicians. Data for these variables had also been collected in the retrospective study. By comparing the results of the two studies, the researcher feels confident in suggesting that the

variables clinicians identified as important, on average, only contained 50.0% of complete male patient data.

The results are in agreement with a 2014 Centers for Disease Control and Prevention consensus which illustrated that the percentage of male data reported to analyze was much less than the amount of female data available. Therefore, it is proposed that analogous research studies be performed to determine an enhanced collection method for these specifically identified variables.

The current study also suggested that there is a notable lack of patient knowledge regarding the importance and contribution of male infertility factors. One possible theory for the reduced amount of male data is that patients are unaware of the actual percentage of the male's reproductive role. Therefore, both males and females might assume it is unnecessary to provide complete information on male patient questionnaires.

Another theory behind the missing data is the sensitive nature of discussing male infertility. Researchers have explained that male patients appeared to be more likely to confide in and desire information and emotional support from infertility clinicians rather than from friends or mental health professionals (Glover et al., 1994; Hammarberg et al., 2010; Brucker and McKenry, 2004). Therefore, if patients are not getting adequate information on male infertility factors from their doctor visits they are highly unlikely to learn about fertility issues and lifestyle exposure factors through additional resources.

Results suggest that forthcoming studies use a combined method to increase data collection, while providing increased patient knowledge on male

infertility variables. For example, future clinical research surveys should be more specific by individually labeling male reproductive threats. The proposed design would also include the specific effects of each of these threats on male reproduction.

Exploratory studies are one suggested design to further analyze the issue of missing male data. Performing a retrospective study would help to identify the response rate but would limit further analysis. A prospective clinical study could also be designed to identify the response rate and then follow up with the male patients on the reasons behind the missing data.

Although the researcher expected this finding, at such a large scope was not intentional. The study was designed to protect for this by collecting as many variables as possible; in the case that some might not provide a large enough sample size. It was not expected for the researcher to find such a significant amount of missing data. In addition, gathering data from male electronic medical records was extremely inefficient and more time consuming than expected. The original design of the retrospective data included a larger sample and was confounded by the collection process of male data. The results for the clinician survey suggest that professionals in the industry are observing similar response rates from male patients.

Conclusion 1

It was concluded that reproductive infertility clinicians who participated in a voluntary male infertility survey did not demonstrate a significant difference of opinions on topics such as; data availability and gender based ethical treatment

of patients. However, gender and the need of better communication system for patient data was extremely close to having a significant correlation. Further research should definitely be designed to address this relationship. Experimental studies need to be performed on the comparison of male data access in each occupation.

Limitations

The biggest limitation of this study was that the researcher was not in complete control of who was selected to participate. Although the group invited to participate in the survey were professionals in the infertility industry, the researcher was reliant on a third party, EmbryoMail, to verify the legitimacy of their credentials.

Another study limitation was the inability to identify the sample response rate of survey participants, creating an extraneous variable threat. A review of the literature demonstrated that previous survey studies have reported extremely variable response rates. In a European social survey on fertility age limits, Billari et al. (2011) reported a 46.0% response rate from one sample and a 73.0% response rate from another. To overcome this and the fact that a specific group was addressed, the researcher utilized descriptive statistics to prove normal distribution among the sample. In addition, descriptive statistics were used to show no significant difference from the national population. Still, another limitation of descriptive research is that it cannot identify a cause and effect relationship.

As with the retrospective study, survey sample size is a limitation. Although normally distributed, small variances may be hidden by the small sample size studied. Another limitation is that the survey was only exploratory. Further research needs to be performed to investigate qualitative and quantitative clinical studies.

Evaluating the influence of age on reproduction has proven to be extremely difficult and controversial. Conclusions remain vulnerable due to many possible confounding cofactors. Sartorius and Nieschlag (2010) explained that not only do individual subjects age at different rates, but effects of age on male reproduction bay be caused by aging, or by mediators generated secondarily by age-related cofactors.

As long as couples continue to advance with the current trend of delayed childbearing, male infertility factors need to remain on the forefront. The opportunities for research topics in the area are endless. Just as important, is the opportunity to inform and educate reproductive age males on the risks and benefits associated with their personal fertility. Although this is not expected to be easily overcome, the stigma of males and their infertility need to be put aside to better treat the infertility couple.

In a 25 European country survey male and female age limits were equally associated by the opposite sex, when it came to their reproductive capabilities (Billari et al., 2011). Billari and collaborators (2011) suggested one reason for this may be that young people in Europe are more aware of recent medical insight into the biological limits to childbearing for both males and females. Another

reason may be that, for young people, notions of gender equality in their lives may be of greater importance so they apply similar reproductive expectations to women and men alike. For them, it is late parenthood rather than late motherhood that should be avoided for reasons physical or otherwise (Billari et al., 2011). This suggestion is promising for the continued acknowledgement of male infertility factors.

There is a strong need for further research studies in all areas of male reproductive physiology. The results of the current study and the review of literature strongly suggest that this is not just a biological issue. Future research needs to address male infertility in an all-encompassing manner including; societal stigmas, religious beliefs, industry ethics, age limits, and a host of environmental and lifestyle factors.

The design of the study should take into consideration the large amount of patients that will be needed and the excess time required to evaluate records on each patient. The present results suggest that researchers should expect to get data from approximately 25.0% of the male records reviewed. Additional research should focus on describing the nature of the missing data. New collection methods should be attempted in an effort to get as much male data as possible.

The researcher submitted a third study to the LSU IRB as a part of this proposal that has not yet been pursued. The approved study is designed around the creation and real time administration of a clinician survey. The instrument would be similar to the study clinic questionnaire, except that patients would be

administered the instrument by a professional. Information would be collected on specific variables, such as the names of specific exposure chemicals.

In addition, this method could meet a second issue that needs to be addressed, properly providing male infertility information to patients. Couples would be informed on the types of fertility treatments available and what lifestyle factors to be addressed promoting increased male reproductive success.

However, still to be determined is the correct provision of privacy or anonymity for male patients. The results of the current study suggested that there are a number of sensitive questions that receive no response from male patients. Future research would need to determine the best way to get the most accurate and available responses from male patients.

In a systematic review of research concerning patients' perspectives on fertility care, Dancet et al. (2010) demonstrated that only three of the 51 studies had focused specifically on male experiences. The authors concluded that there was a lack of data regarding men's perceptions of care, particularly with regard to invasive procedures (Dancet et al, 2010).

In their 2012 review, Dudgeon and Inhorn, evaluated 92 publications through a search of available literature on the psychological and social aspects of infertility in men. Although psychological and social aspects of infertility, fertility treatment with assisted reproductive technologies, and infertility-related childlessness have been investigated comprehensively in women, the psychosocial consequences of infertility for men are less well understood (Greil et al., 2010). Previously reported in a 2010 publication, Greil and colleagues

explained that most of the participants in each male study were recruited from clinical services and little was known about men who do not seek treatment. The group also pointed out that among those who do in fact pursue treatment; a number of male behavioral factors still remain unknown.

Data gained by the researcher from the current 2015 study supports both statements in the above paragraph. Reflecting the 2010 Greil et al. review, the 2015 retrospective study samples were also comprised of a male patient population that received clinical infertility services. In addition, the researcher's observations from the current study was consistent with Greil and colleagues' 2010 account, confirming that there are still a large number of unknowns and incomplete information available for males who do participate in clinical services.

There are a number of past and present reasons that have led to the lack of male patient information. However, results from the current study led the researcher to focus on two of the possible current issues; male fertility stigma and lack of contribution knowledge.

Based on their review of biological and cultural anthropological theories on masculinity and human reproduction, Dudgeon and Inhorn (2012) concluded that male infertility is more stigmatizing for men than it is for women. The authors argued that infertility is potentially humiliating and emasculating to many men. As a male, any association with infertility, virility, and sexual potency can lead to perceived personal inadequacy (Dudgeon and Inhorn, 2012).

In Sweden, Hjelmstedt et al. (1999) found that 50.0% more men, than women had not shared their infertility problems with others. The authors

interpreted these results as reflecting the inherent frustrations of being in a situation that is poorly understood and in which assured treatments are not guaranteed (Hjelmstedt et al., 1999). While there is an emerging body of evidence focused on the psychological and social aspects of infertility for men, significant knowledge gaps remain (Greil, et al., 2010; Sherrod, 2006).

The current study results provided valuable information for further research. Additionally, some of the results can be utilized by fertility clinicians and infertility patients participating in clinical ART treatment cycles. These results also support the growing body of research that is examining the effect of advanced paternal age on male reproductive success.

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APPENDIX A: IRB RETROSPECTIVE STUDY APPROVAL FORM

ACTION ON EXEMPTION APPROVAL REQUEST

Institutional Review Board
Dr. Dennis Landin, Chair
130 David Boyd Hall
Baton Rouge, LA 70803
P: 225.578.8692
F: 225.578.5983
irb@lsu.edu | lsu.edu/irb

TO: Jeanne Glaser
Human Resource Education

FROM: Dennis Landin
Chair, Institutional Review Board

DATE: August 28, 2014

RE: **IRB#** E8892

TITLE: A Retrospective Study: Examining the Male Infertility Factor; The Association Between Age, Environment and Reproductive Success

New Protocol/Modification/Continuation: New Protocol

Review Date: 8/27/2014

Approved X **Disapproved**_____

Approval Date: 8/27/2014 **Approval Expiration Date:** 8/26/2017

Exemption Category/Paragraph: 4a

Signed Consent Waived?: Yes

Re-review frequency: (three years unless otherwise stated)

LSU Proposal Number (if applicable): _____

Protocol Matches Scope of Work in Grant proposal: (if applicable) _____

By: Dennis Landin, Chairman _____

PRINCIPAL INVESTIGATOR: PLEASE READ THE FOLLOWING –
Continuing approval is CONDITIONAL on:

1. Adherence to the approved protocol, familiarity with, and adherence to the ethical standards of the Belmont Report, and LSU's Assurance of Compliance with DHHS regulations for the protection of human subjects*
2. Prior approval of a change in protocol, including revision of the consent documents or an increase in the number of subjects over that approved.
3. Obtaining renewed approval (or submittal of a termination report), prior to the approval expiration date, upon request by the IRB office (irrespective of when the project actually begins); notification of project termination.
4. Retention of documentation of informed consent and study records for at least 3 years after the study ends.
5. Continuing attention to the physical and psychological well-being and informed consent of the individual participants, including notification of new information that might affect consent.
6. A prompt report to the IRB of any adverse event affecting a participant potentially arising from the study.
7. Notification of the IRB of a serious compliance failure.
8. SPECIAL NOTE:

**All investigators and support staff have access to copies of the Belmont Report, LSU's Assurance with DHHS, DHHS (45 CFR 46) and FDA regulations governing use of human subjects, and other relevant documents in print in this office or on our World Wide Web site at http://www.lsu.edu/irb*

APPENDIX B: IRB APPROVED PATIENT CONSENT FORM WAIVER

Consent Form Script: Retrospective Study
(IRB waived consent form)

1. Study Title: A Retrospective Study: Examining the Male Infertility Factor; The Association Between Male Age, Environment and Reproductive Success

2. Performance Site: Louisiana State University and Agricultural and Mechanical College

3. Investigators: The following investigators are available for questions about this study,
M-F, 8:00 a.m. - 4:30 p.m.
Jeanne L. Glaser, MS
jglase1@lsu.edu

4. Purpose of the Study: The purpose of this study is to determine whether there is an association between gender, age and environment as they relate to reproductive success.

5. Subject Inclusion: Individuals over the age of 18 who have participated in assisted reproductive technology.

6. Number of subjects: 250

7. Study Procedures: A retrospective study will be conducted to determine the association between the age of a couple, environmental factors and reproductive success. Patient information will be collected from participating human reproductive clinics. Patient anonymity will be maintained at all phases of data collection and analysis.

8. Benefits: The study may yield valuable information about the association of gender, age and environment as they relate to reproductive success.

9. Risks: The only study risk is the inadvertent release of sensitive information found in patient medical records. However, every effort will be made to maintain the anonymity of study records. All identifying patient factors will be removed from the specific clinic computer used to transfer study data to the researcher. In addition, the computer Wi-Fi connection will be disconnected before the USB flash drive is inserted to save files.

10. Right to Refuse: Subjects may choose not to participate or to withdraw from the study at any time without penalty or loss of any benefit to which they might otherwise be entitled.

11. Privacy: Results of the study may be published, but no names or identifying information will be included in the publication. Subject identity will remain confidential unless disclosure is required by law.

12. Signatures:

The study has been discussed with me and all my questions have been answered. I may direct additional questions regarding study specifics to the investigators. I agree to participate in the study described above and acknowledge the investigator's obligation to provide me with a signed copy of this consent form.

This study has been approved by the LSU IRB. For questions concerning participant’s rights, please contact the IRB Chair, Dr. Dennis Landin, Institutional Review Board, (225) 578-8692, irb@lsu.edu, www.lsu.edu/irb.

APPENDIX C: RESEARCH CONFIDENTIALITY AGREEMENT

CONFIDENTIALITY AND NONDISCLOSURE AGREEMENT

THIS CONFIDENTIALITY AND NONDISCLOSURE AGREEMENT (the "**Agreement**") is made and entered into as of _____ between [redacted] ("**Company**") and **Jeanne L. Glaser, MS** ("**Researcher**").

1. PURPOSE

Company and Researcher wish to collaborate in a retrospective patient research study. Company may disclose to Researcher certain confidential technical and patient information which Company desires Researcher to treat as confidential.

2. CONFIDENTIAL INFORMATION

"**Confidential Information**" means any information disclosed to Researcher by Company, either directly or indirectly in writing, orally or by inspection of tangible objects, including without limitation patient records or personal information of patients, electronic records, images, ownership information. Confidential Information shall also include without limitation the items set forth in the Appendix attached hereto.

3. NON-USE AND NON-DISCLOSURE

Researcher agrees not to use any Confidential Information for any purpose except to fulfill retrospective research data requirements as listed in Appendix. In addition, specific measures will be taken to protect patient anonymity and all records in Researcher's possession will be destroyed at the completion of the study. Researcher shall not use any Confidential Information in any manner detrimental to the business interests of Company. Researcher agrees not to disclose any Confidential Information to any third parties. Researcher shall not disclose Confidential Information to advisors of Researcher.

4. MAINTENANCE OF CONFIDENTIALITY

Researcher agrees that it shall take all reasonable measures to protect the secrecy of and avoid disclosure and unauthorized use of the Confidential Information. Without limiting the foregoing, Researcher shall take at least those measures that Researcher takes to protect its own most highly confidential information. Researcher shall not make any copies of Confidential Information unless the same are previously approved in writing by the Company. Researcher shall reproduce Company's proprietary rights notices on any such approved copies, in the same manner in which such notices were set forth in or on the original. Researcher shall immediately notify Company in the event of any unauthorized use or disclosure of the Confidential Information.

5. NO OBLIGATION

Nothing herein shall obligate Company or Researcher to proceed with any transaction between them, and each party reserves the right, in its sole discretion, to terminate the discussions contemplated by this Agreement concerning the business opportunity.

6. NO WARRANTY

ALL CONFIDENTIAL INFORMATION IS PROVIDED "AS IS." COMPANY MAKES NO WARRANTIES, EXPRESS, IMPLIED OR OTHERWISE, REGARDING ITS ACCURACY, COMPLETENESS OR PERFORMANCE.

7. RETURN OF MATERIALS

All documents and other tangible objects containing or representing Confidential Information and all copies thereof which are in the possession of Researcher shall be and remain the property of Company and tangible objects shall be promptly returned to Company. All electronic patient records will be destroyed by Researcher.

8. NO LICENSE

Nothing in this Agreement is intended to grant any rights to Researcher under any patent, mask work right or copyright of Company, nor shall this Agreement grant Researcher any rights in or to Confidential Information except as expressly set forth herein.

9. TERM

This Agreement shall survive indefinitely, not just upon the completion of the study.

10. REMEDIES

Researcher agrees that any violation or threatened violation of this Agreement will cause irreparable injury to the Company, entitling Company to obtain injunctive relief in addition to all legal remedies.

11. MISCELLANEOUS

This Agreement shall bind and inure to the benefit of the parties hereto and their successors and assigns, except that Researcher may not assign or transfer this Agreement, by operation of law or otherwise, without Company's prior written consent. THIS AGREEMENT SHALL BE GOVERNED BY THE LAWS OF THE STATE OF LOUISIANA, WITHOUT REFERENCE TO CONFLICT OF LAWS matter hereof. If any provision of this Agreement is found to be illegal or unenforceable, the other provisions shall remain effective and enforceable to the greatest extent permitted by law. Any failure to enforce any provision of this Agreement shall not constitute a waiver thereof or of any other provision hereof. This Agreement may not be amended, nor any obligation waived, except by a writing signed by both parties hereto. The parties may execute this Agreement in counterparts, each of which is deemed an original, but all of which together constitute one and the same agreement.

ACCEPTED AND AGREED TO BY THE AUTHORIZED REPRESENTATIVE OF EACH PARTY:

COMPANY:	**RESEARCHER:**
[redacted]	**Jeanne L. Glaser, MS**
Signature: _____	Signature: _____
Title: _____	University: _____
Date Signed:_____	Title: _____
	Date Signed:_____

Appendix

List of other additional, particular items subject to confidentiality and non-disclosure:

1. See LSU Institutional Review Board Action on Exemption Approval Request.

2. See Brief Summary of the Project, A Retrospective Study: Examining the Male Infertility Factor; The Association Between Age, Environment and Reproductive Success.

3. See LSU Institution Review Board Application for Exemption from Institutional Oversight.

4. See Consent Form Script: Retrospective Study (IRB waived consent form).

APPENDIX D: IRB CLINICIAL SURVEY APPROVAL FORM

ACTION ON EXEMPTION APPROVAL REQUEST

Institutional Review Board
Dr. Dennis Landin, Chair
130 David Boyd Hall
Baton Rouge, LA 70803
P: 225.578.8692
F: 225.578.5983
irb@lsu.edu | lsu.edu/irb

TO: Jeanne Glaser
Human Resource Education

FROM: Dennis Landin
Chair, Institutional Review Board

DATE: August 19, 2014

RE: **IRB#** E8894

TITLE: Clinician Survey: Examining the Male Infertility Factor; The Association Between Age, Environment and Reproductive Success

New Protocol/Modification/Continuation: New Protocol

Review Date: 8/18/2014

Approved_____X_____ **Disapproved**_____

Approval Date: 8/18/2014 **Approval Expiration Date:** 8/17/2017

Exemption Category/Paragraph: 2a,b

Signed Consent Waived?: Yes

Re-review frequency: (three years unless otherwise stated)

LSU Proposal Number (if applicable): _____

Protocol Matches Scope of Work in Grant proposal: (if applicable) _____

By: Dennis Landin, Chairman _____

PRINCIPAL INVESTIGATOR: PLEASE READ THE FOLLOWING –
Continuing approval is CONDITIONAL on:

1. Adherence to the approved protocol, familiarity with, and adherence to the ethical standards of the Belmont Report, and LSU's Assurance of Compliance with DHHS regulations for the protection of human subjects*
2. Prior approval of a change in protocol, including revision of the consent documents or an increase in the number of subjects over that approved.
3. Obtaining renewed approval (or submittal of a termination report), prior to the approval expiration date, upon request by the IRB office (irrespective of when the project actually begins); notification of project termination.
4. Retention of documentation of informed consent and study records for at least 3 years after the study ends.
5. Continuing attention to the physical and psychological well-being and informed consent of the individual participants, including notification of new information that might affect consent.
6. A prompt report to the IRB of any adverse event affecting a participant potentially arising from the study.
7. Notification of the IRB of a serious compliance failure.
8. SPECIAL NOTE:

**All investigators and support staff have access to copies of the Belmont Report, LSU's Assurance with DHHS, DHHS (45 CFR 46) and FDA regulations governing use of human subjects, and other relevant documents in print in this office or on our World Wide Web site at http://www.lsu.edu/irb*

APPENDIX E: IRB APPROVED CLINICIAN SURVEY

IRB Approved Male Infertility Clinician Survey

Please feel free to skip any question(s) that does not apply to you and only respond to the questions that are related to your professional experience. Any input is greatly appreciated.

1. **What role do you play in reproductive health care?**

- ○ Reproductive Endocrinologist, MD
- ○ Reproductive Physiologist, PhD
- ○ Andrologist
- ○ Embryologist
- ○ Urologist
- ○ OB/GYN
- ○ Researcher
- ○ Reproductive Technician

Other (please specify)

2. Gender

- ○ Male
- ○ Female

3. What descriptive personal data does your facility obtain from male infertility patients? (Check all that apply.)

- [ ] Age
- [ ] Race
- [ ] Height
- [ ] Weight
- [ ] BMI
- [ ] Fathered Pregnancy with Current Female Partner
- [ ] Fathered Pregnancy with Non-Current Female Partner
- [ ] Infertility Length with Current Partner

Other (please specify)

4. **In your current position, do you have access to and review all descriptive data for each male patient per cycle?**

- ( ) Always
- ( ) Mostly
- ( ) Occasionally
- ( ) Rarely
- ( ) Never

5. **In your professional opinion, what are the most commonly seen descriptive characteristics that you observe in correlation to male infertility? (You may select more than one.)**

- [ ] Age
- [ ] Race
- [ ] Height
- [ ] Weight
- [ ] BMI
- [ ] Infertility Length with Current Partner

Other (please specify)

6. What semen parameters does your facility analyze in male infertility patients? (Check all that apply.)

- [ ] Volume
- [ ] Sperm Count
- [ ] Sperm Motility
- [ ] Progressive Motility
- [ ] Sperm Morphology
- [ ] White Blood Cell Count
- [ ] Acrosome Integrity
- [ ] DNA Fragmentation

Other (please specify)

7. In your current position, do you have access to and review all semen data for each male patient per cycle?

- ( ) Always
- ( ) Mostly
- ( ) Occasionally
- ( ) Rarely
- ( ) Never

8. In your professional opinion, what are the most commonly seen semen parameters that you observe in correlation to male infertility? (You may select more than one.)

- [ ] Volume
- [ ] Sperm Count
- [ ] Sperm Motility
- [ ] Progressive Motility
- [ ] Sperm Morphology
- [ ] White Blood Cell Count
- [ ] Acrosome Integrity
- [ ] DNA Fragmentation

Other (please specify)

9. Is basic semen analysis (morphology, motility, concentration) predictive of male infertility?

- ○ Always
- ○ Mostly
- ○ Occasionally
- ○ Rarely
- ○ Never
- ○ No Idea

10. Is DNA fragmentation predictive of male infertility?

- ○ Always
- ○ Mostly
- ○ Occasionally
- ○ Rarely
- ○ Never
- ○ No Idea

11. How significantly do you think male age contributes to impaired semen and/or sperm cells?

- ○ A Little
- ○ Somewhat
- ○ A Lot
- ○ Completely Responsible
- ○ No Idea

12. How significantly do you think genetic/epigenetic changes in sperm DNA contribute to male infertility?

- ○ A Little
- ○ Somewhat
- ○ A Lot
- ○ Completely Responsible
- ○ No Idea

13. What additional tests do you currently use to evaluate semen parameters?

14. Where do these tests fall short?

15. What urological data does your facility obtain from male infertility patients? (Check all that apply.)

- [ ] History of Previous Urologist Visit
- [ ] Impotence
- [ ] Unable to Impregnate Female Partner
- [ ] Sperm Donor History
- [ ] Hormonal Treatment
- [ ] Vasectomy
- [ ] Surgery to Testicles
- [ ] Undescended Testicles
- [ ] Testicular Trauma
- [ ] Testicular Swelling
- [ ] Testicular Torsion
- [ ] History of White Blood Cells in Semen
- [ ] History of Prostate Infection

Other (please specify)

16. In your current position, do you have access to and review all urological data for each male patient per cycle?

- ( ) Always
- ( ) Mostly
- ( ) Occasionally
- ( ) Rarely
- ( ) Never

17. In your professional opinion, what are the most commonly seen urological issues that you observe in correlation with male infertility? (You may select more than one and/or list others.)

- [ ] Impotence
- [ ] Hormonal Treatment
- [ ] Vasectomy
- [ ] Surgery to Testicles
- [ ] Undescended Testicles
- [ ] Testicular Trauma
- [ ] Testicular Swelling
- [ ] Testicular Torsion
- [ ] History of White Blood Cells in Semen
- [ ] History of Prostate Infection

Other (please specify)

18. What exposure(s) or environmental factor(s) data does your facility obtain from male infertility patients? (Check all that apply.)

- [ ] Occupation
- [ ] Smoking (Self or Partner)
- [ ] Diet
- [ ] Alcoholic Drinks
- [ ] Number of Caffeinated Beverages
- [ ] Recreational Drugs
- [ ] Bath/Hot Tub Use
- [ ] Recent High Fever
- [ ] Steroids for Body Building
- [ ] Exposure to Chemicals

Other (please specify)

19. In your current position, do you have access to and review all exposure and environmental data in each male patient per cycle?

- ○ Always
- ○ Mostly
- ○ Occasionally
- ○ Rarely
- ○ Never

20. How significantly do you think male exposure(s) and environmental factor(s) contribute to impaired semen and/or sperm cells?

- ○ A Little
- ○ Somewhat
- ○ A Lot
- ○ Completely Responsible
- ○ No Idea

21. In your professional opinion, what are the most commonly seen exposures that you observe in correlation with male infertility? (You may select more than one and/or list others.)

- ☐ Occupation
- ☐ Smoking (Self or Partner)
- ☐ Diet
- ☐ Alcoholic Drinks
- ☐ Number of Caffeinated Beverages
- ☐ Recreational Drugs
- ☐ Bath/Hot Tub Use
- ☐ Recent High Fever
- ☐ Steroids for Body Building
- ☐ Exposure to Chemicals

Other (please specify)

22. What percentage of male patients do you have complete medical records on their personal environmental and exposure factors?

- ○ Less than 25%
- ○ 25% to 49%
- ○ 50%
- ○ 51% to 75%
- ○ Greater than 75%

23. Would access to more male patient lifestyle information improve your ability to provide better care?

- ○ Yes
- ○ No
- ○ Undecided

24. What medical history data does your facility obtain from male infertility patients? (Check all that apply.)

- ☐ BMI
- ☐ Blood Pressure
- ☐ Medication Usage (Prescription and/or Herbal)
- ☐ Recent Illness/Infection
- ☐ Birth Defects
- ☐ Participated in Prior Fertility Treatment
- ☐ Ancestry of Mother and Father

Other (please specify)

25. In your current position, do you have access to and review all medical history data in each male patient per cycle?

- ○ Always
- ○ Mostly
- ○ Occasionally
- ○ Rarely
- ○ Never

26. In your professional opinion, what are the most commonly seen medical data characteristics that you observe in correlation with male infertility? (You may select more than one and/or list others.)

- [ ] BMI
- [ ] Blood Pressure
- [ ] Medication Usage (Prescription and/or Herbal)
- [ ] Recent Illness/Infection
- [ ] Birth Defects
- [ ] Ancestry of Mother and Father

Other (please specify)

27. In addition to basic semen analysis, what tests do you currently use to diagnose male infertility?

28. Where do these tests fall short?

29. Where do you think the problem originates with impaired sperm cells? (You may select more than one and/or list others.)

- [ ] Germ Cell Stage
- [ ] DNA Fragmentation
- [ ] Epigenetics
- [ ] Spermatogenesis
- [ ] Oxidation/ROS
- [ ] Endocrine Disruptors
- [ ] Other (please specify)

30. Does reproductive healthcare need a better diagnostic test for male factor infertility or are the currently available tests sufficient?

- ○ Need Better Test
- ○ Current Tests Are Sufficient
- ○ Undecided

31. In your professional opinion, would further diagnostic semen testing in the clinical setting be significant enough to justify additional upfront costs and time for a more efficient treatment plan.

- Yes
- No
- Undecided

32. Does reproductive healthcare need a better communication system allowing the physician and the laboratory physiologist to have equal access to the all of the patient information per cycle (including all of the characteristics mentioned above)?

- Need Better System
- Current System Is Sufficient
- Undecided

33. As you may be aware some clinics have a female egg cutoff age, does your clinic have a male sperm cutoff age?

- Yes
- No

If yes what age?

34. In your professional opinion do you find it unethical to provide fertility treatment for males after a certain age?

- Yes
- No

If yes what age?

35. In your professional opinion, do you find that we are adequately providing significant clinical information to older male patients on the risks and ethical issues of advanced age fertility treatments?

- Yes
- No
- Undecided

36. In your professional opinion, clinical reproductive infertility cycle failures are due to:
(Please just list percentage numbers)

(1-100%) Female Infertility

(1-100%) Male Infertility

(1-100%) Combination of Male and Female Infertility

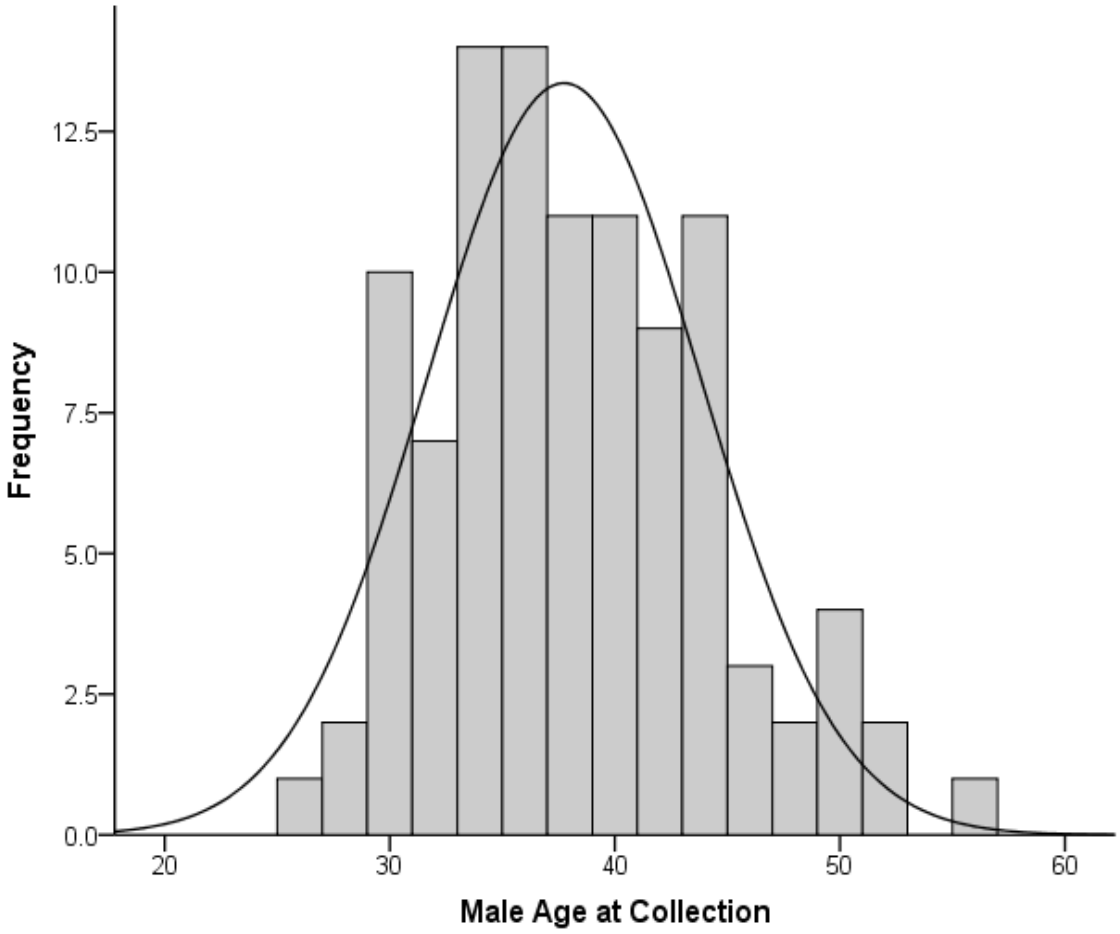
(1-100%) Unknown Infertility

Done

Research Survey Created by Jeanne L. Glaser, LSU AgCenter 2015

APPENDIX F: IBM SPSS OUTPUT

1. Male age at collection frequency normal distribution curve.



2. One-sample t-test analysis of the retrospective study sample average pregnancy rate (44.0%) compared to the national ART pregnancy rate average (39.0%).

One-Sample T-Test

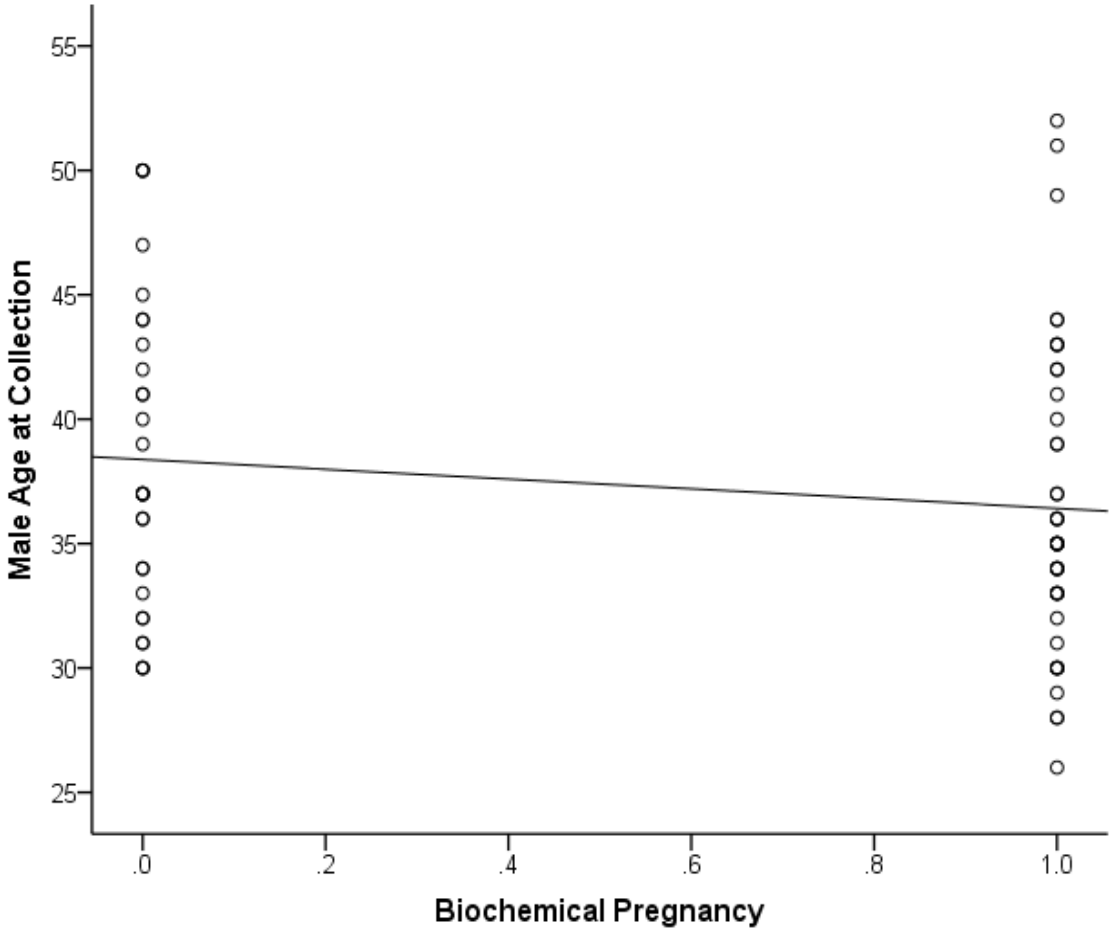
	National ART Pregnancy Rate Average = .39					
					95.0% Confidence Interval of the Difference	
	t	Df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Biochemical Pregnancy	1.036	101	.303	.051	-.05	.15

3. One-sample t-test analysis of the retrospective study sample average male BMI level (29) compared to the national average male BMI level (29).

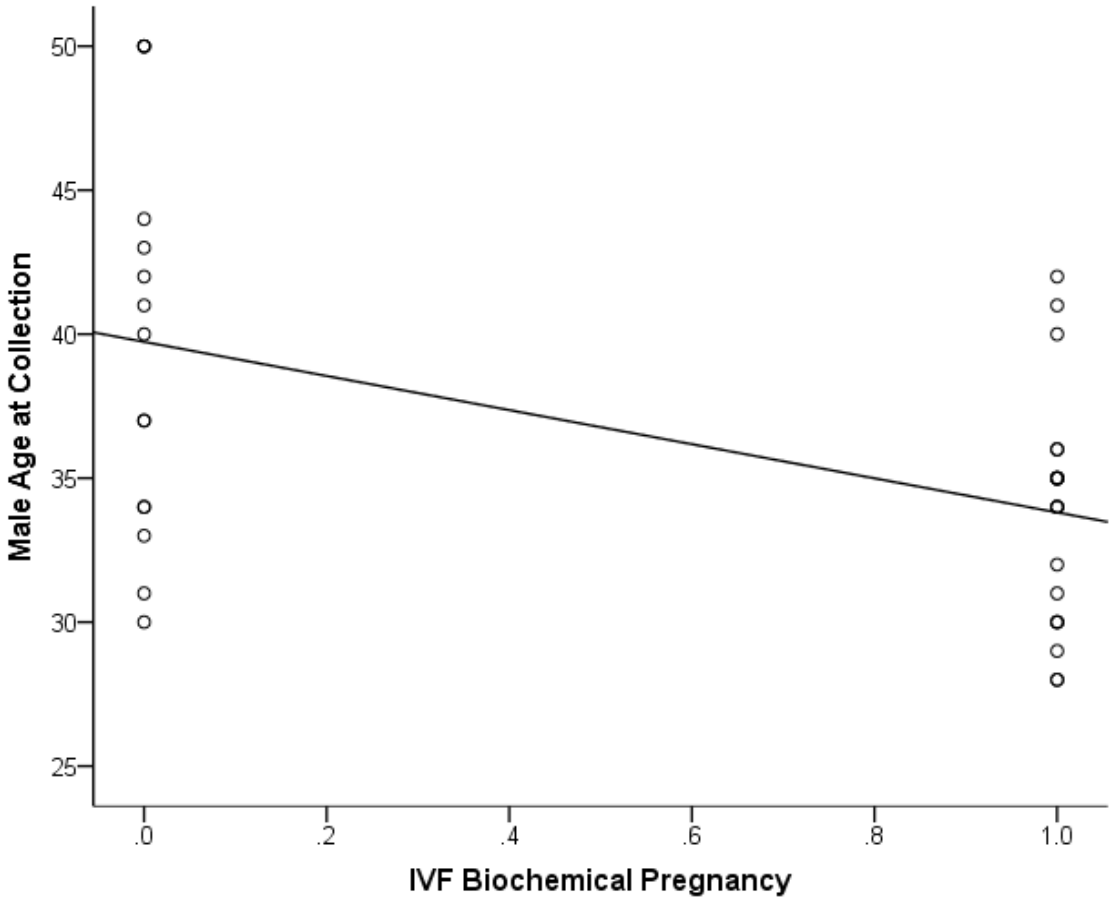
One-Sample T-Test

	National Male BMI Average = 29					
					95.0% Confidence Interval of the Difference	
	t	Df	Sig. (2-tailed)	Mean Difference	Lower	Upper
BMI	-.633	49	.530	-.49180	-2.0525	1.0689

4. Point-biserial correlation coefficient scatter/dot plot exhibiting negative relationship between biochemical pregnancy and male age.



5. Point-biserial correlation coefficient scatter/dot plot exhibiting negative relationship between IVF biochemical pregnancy and male age.

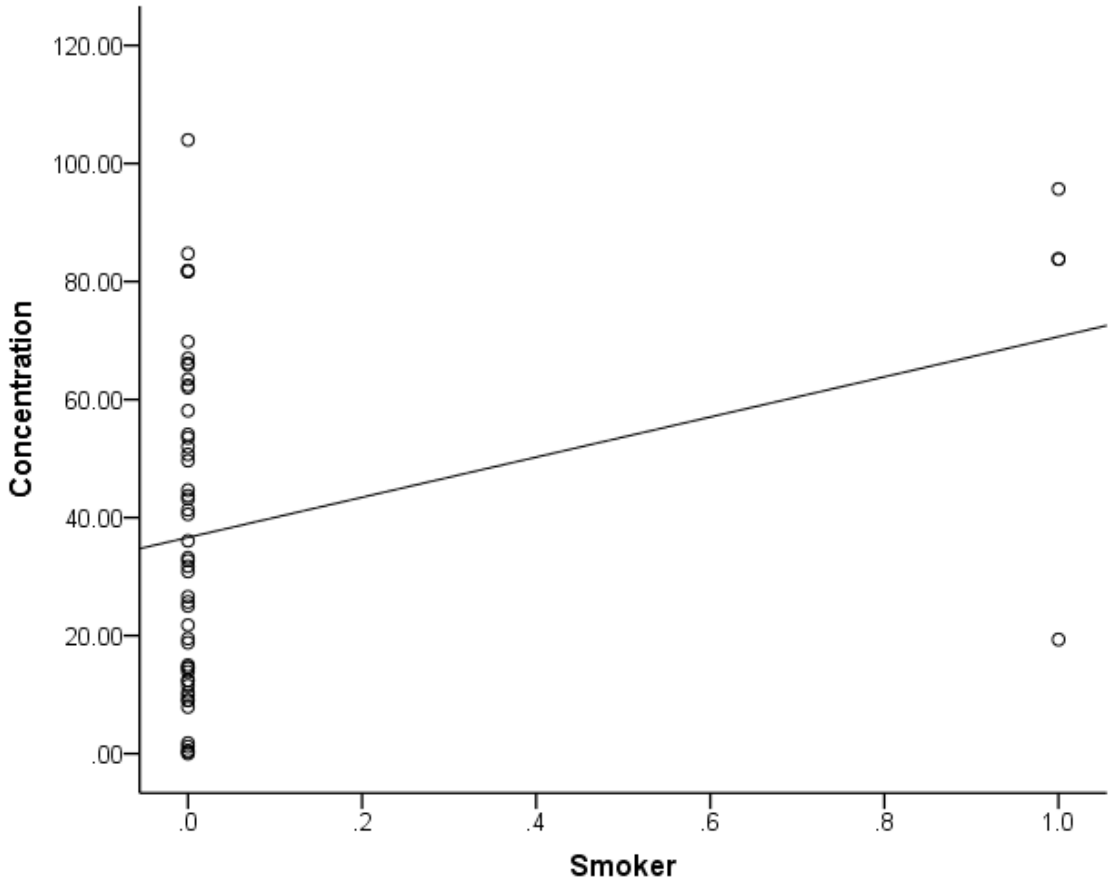


6. Retrospective male patient alcohol usage.

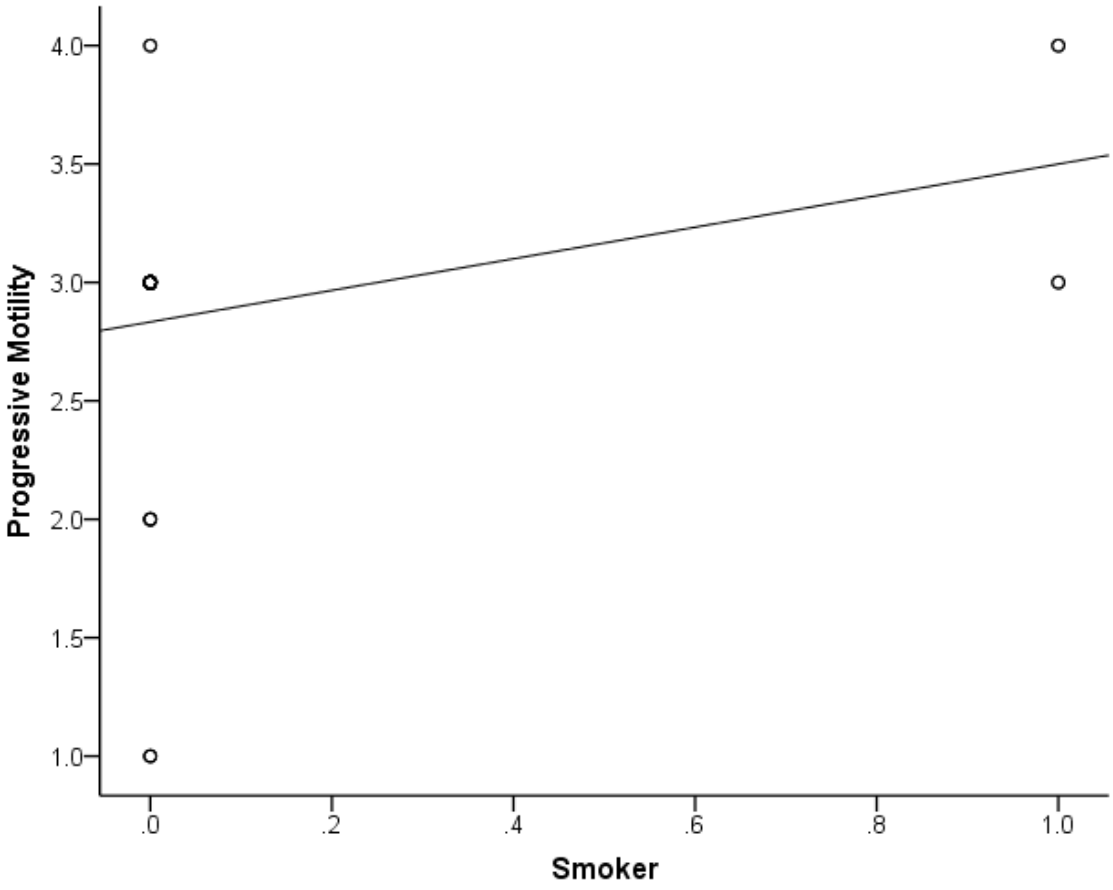
Descriptive Statistics

	Mean	Std. Deviation	N
Social Alcohol Usage	.6250	.48925	48
Volume	2.429	1.6007	83
Total Motile Sperm/Specimen	47.1173	50.60242	83

7a. Point-biserial correlation coefficient scatter/dot plot exhibiting negative relationship between smoker and semen concentration.



7b. Point-biserial correlation coefficient scatter/dot plot exhibiting negative relationship between smoker and semen progressive motility.



8.

Would access to more male patient lifestyle information improve your ability to provide better care?	Response Percent	Response Count
Yes	53.8%	28
No	19.2%	10
Undecided	26.9%	14
Total		52

9.

Does reproductive healthcare need a better communication system allowing the physician and the laboratory physiologist to have equal access to all of the patient information per cycle?	Response Percent	Response Count
Need a better system	53.8%	28
Current system is sufficient	42.3%	22
Undecided	3.9%	2
Total		52

10a.

Occupation*Descriptive Data Access Contingency Table

	Descriptive Data Access				
Occupation	Always	Mostly	Occasionally	Rarely	Total
Repro Endo	3	0	0	0	3
Repro Phys	4	1	4	4	13
Embryologist	17	7	3	3	30
Andrologist	4	1	0	0	5
Urologist	1	0	0	0	1
Reproductive Tech	0	0	0	1	1
Total	29	9	7	8	53

10b.

Cramer's V Correlation Coefficient Occupation*Descriptive Data Access		Value	Approx. Sig.
Nominal by Nominal	Phi	.608	.187
	Cramer's V	.351	.187
N of Valid Cases		53	

11a.

Occupation*Semen Data Access Contingency Table

	Semen Data Access					
Occupation	Always	Mostly	Occasionally	Rarely	Never	Total
Repro Endo	3	0	0	0	0	3
Repro Phys	8	3	1	0	1	13
Embryologist	20	8	0	1	1	30
Andrologist	5	0	0	0	0	5
Urologist	1	0	0	0	0	1
Reproductive Tech	0	1	0	0	0	1
Total	37	12	1	1	2	53

11b.

Cramer's V Correlation Coefficient Occupation*Semen Data Access		Value	Approx. Sig.
Nominal by Nominal	Phi	.470	.926
	Cramer's V	.235	.926
N of Valid Cases		53	

12a.

Occupation*Urology Data Access Contingency Table

Occupation	Urology Data Access					
	Always	Mostly	Occasionally	Rarely	Never	Total
Repro Endo	2	0	1	0	0	3
Repro Phys	4	1	7	1	0	13
Embryologist	10	8	6	3	1	28
Andrologist	1	1	3	0	0	5
Urologist	1	0	0	0	0	1
Reproductive Tech	0	0	0	1	0	1
Total	18	10	17	5	1	51

12b.

Cramer's V Correlation Coefficient Occupation*Urology Data Access		Value	Approx. Sig.
Nominal by Nominal	Phi	.634	.427
	Cramer's V	.317	.427
N of Valid Cases		51	

13a.

Occupation*Exposure Data Access Contingency Table

	Exposure Data Access					
Occupation	Always	Mostly	Occasionally	Rarely	Never	Total
Repro Endo	2	0	1	0	0	3
Repro Phys	3	2	4	4	0	13
Embryologist	8	8	6	6	1	29
Andrologist	2	1	2	0	0	5
Urologist	1	0	0	0	0	1
Reproductive Tech	0	0	0	1	0	1
Total	16	11	13	11	1	52

13b.

Cramer's V Correlation Coefficient Occupation*Exposure Data Access		Value	Approx. Sig.
Nominal by Nominal	Phi	.501	.874
	Cramer's V	.251	.874
N of Valid Cases		52	

14a.

Occupation*Medical Data Access Contingency Table

	Medical Data Access					
Occupation	Always	Mostly	Occasionally	Rarely	Never	Total
Repro Endo	2	0	1	0	0	3
Repro Phys	3	0	4	5	1	13
Embryologist	10	7	7	2	2	28
Andrologist	1	1	2	0	0	4
Urologist	1	0	0	0	0	1
Reproductive Tech	0	0	1	0	0	1
Total	17	8	15	7	3	50

14b.

Cramer's V Correlation Coefficient Occupation*Medical Data Access		Value	Approx. Sig.
Nominal by Nominal	Phi	.613	.537
	Cramer's V	.306	.537
N of Valid Cases		50	

VITA

Jeanne Lee Glaser was born to Jaqueline C. Martin and Theodore H. Glaser III, along with a younger brother R. Jude Glaser and a younger sister Kimberly R. Glaser. Jeanne is married to Jason Albert Higgins, a board certified sports orthopedic surgeon and a graduate of Louisiana State University Medical School. The couple resides in Thibodaux, Louisiana. The two have Bachelor of Science degrees from Louisiana State University, Baton Rouge, LA. In 2007, Jeanne also graduated with a Master of Science degree, under the guidance of her Major Professor, Dr. Robert A. Godke, Boyd Professor of Reproductive Physiology at Louisiana State University, Baton Rouge, Louisiana. Mrs. Glaser has been employed with the Louisiana State University Agriculture Center since beginning her master's degree program in 2004. During this time she has served as a graduate assistant, interim cooperate relations associate, and extension agent. After, thoughtful consideration Jeanne decided to return to LSU in 2010 to complete a PhD in Human Resource Development with a minor in Reproductive Physiology under the guidance of Dr. William Richardson, Dr. Michael Burnett, Dr. Kenneth Bondioli, and Dr. Robert Godke. Jeanne is a member of several professional memberships including; The International Embryo Transfer Society, The American Society of Reproductive Medicine, The National Society of Collegiate Scholars (Honor Society), Gamma Sigma Delta Honor Society of Agriculture, and Omicron Delta Kappa. While attending Louisiana State University, Jeanne was inducted to The National Dean's List and received the Emily Fairchild Memorial Scholarship award. Mrs. Glaser is a candidate for the

Doctorate of Philosophy degree in Human Resource Education and Workforce Development with a concentration in Reproductive Physiology.